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(71) Applicant *(for all designated States except US)*: CURAGEN CORPORATION [US/US]; 11th floor, 555 Long Wharf Drive, New Haven, CT 06511 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: NANDABALAN, Krishnan [US/US]; 228 Village Pond Road, Guilford, CT 06437 (US). YANG, Meija [US/US]; 6 Catbird Lane, East Lyme, CT 06333 (US). SCHULZ, Vincent, Peter [US/US]; 21 Old Farms Road, Madison, CT 06443 (US).

(74) Agent: ELRIFI, Ivor, R.; Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P. C., One Financial Center, Boston, MA 02111 (US).

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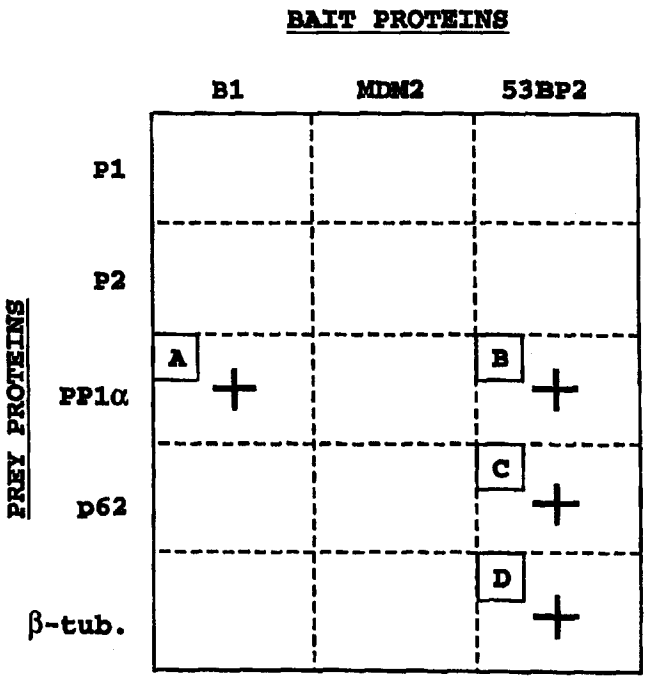
(54) Title: 53BP2 COMPLEXES

(57) Abstract

The present invention discloses complexes of the 53BP2 protein with proteins identified as interacting with 53BP2 (53BP2-IPs) by a yeast two hybrid assay system. The proteins which were demonstrated to interact with 53BP2 are: β-tubulin, p62, hnRNP G, and three gene products, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3, which are encoded (in part) by the EST R72810 sequence. Thus, the present invention discloses complexes of 53BP2 and β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3, and derivatives, fragments and analogs thereof. The present invention also discloses the 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 genes and proteins, as well as derivatives, fragments and analogs thereof. Methodologies for screening the aforementioned complexes for efficacy in treating and/or preventing certain diseases and disorders (particularly, cancer, autoimmune disease and neurodegenerative disease) are also provided by the present invention.



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53BP2 COMPLEXES

RELATED PATENT APPLICATIONS AND GRANT SUPPORT

This application claims priority to United States Utility Patent Application Serial No. 08/935,450, originally filed on September 23, 1997, which is entitled "53BP2 COMPLEXES" and is hereby incorporated in its entirety by reference herein.

The invention disclosed herein was made with United States Government support under award number 70NANB5H1066 awarded by the National Institute of Standards and Technology. Accordingly, the United States Government has certain rights in the present invention.

FIELD OF THE INVENTION

The field of the present invention relates to the complexes of the 53BP2 protein with various other proteins, including, but not limited to, complexes of the 53BP2 protein with β-tubulin, p62, hnRNP G, IP-l, IP-2 and IP-3 proteins. The present invention also relates to the production of antibodies specific for complexes of the 53BP2 protein and the aforementioned proteins, and their use in, *inter alia*, screening, diagnosis, prognosis and therapy. Additionally, the present invention further relates to 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 genes and proteins, as well as derivatives, fragments and analogs, thereof.

BACKGROUND OF THE INVENTION

It should be noted that the novel complexes of the present invention, which will be disclosed *infra*, comprising complexes of the 53BP2 protein with β-tubulin, p62, hnRNP G, IP-1, IP-2, or IP-3 have heretofore not been described. Accordingly, citation of a reference herein shall not be construed as an admission that such is prior art to the present invention.

(1) THE 53BP2 PROTEIN

The human Bcl 2/p53 binding protein, also known as 53BP2, or BEP [GenBank Acc. Number U58334] has been demonstrated to impede cell cycle progression from G2 to M phase, as well directly competing with Bcl 2 for binding to p53, a critical tumor suppressor protein

Introduction (W.H. Freeman and Co., New York, NY). Accordingly, 53BP2 serves a critical role in the modulation of p53 function and thus, mediates cell cycle progression. See e.g., Naumovski & Cleary, 1996. *Mol. Cell. Biol.* 16:3884-3892.

The 53BP2 protein has been shown to bind to the central DNA binding domain of p53 via two adjacent ankryin repeats and an 5H3 domain, thus supporting its ability to modulate, *inter alia*, the DNA binding-ability and stability of the p53 protein and thus, its tumor suppression functions. See e.g., Iwabuchi, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 6098-6102). With respect to the genesis of human neoplasia, the most frequent, observed p53 mutations maps to the 53BP2 binding domain. See e.g., Gorina & Pavletich, 1996. *Science* 274:1001-1005). 53BP2 also modulates the dephosphorylation status (and thus the function of p53) by the binding of protein phosphatase 1 (PPl) to the carboxyl-terminal region of 53BP2 containing the aforementioned, critical ankyrin and 5H3 binding domains. See e.g., Helps, *et al.*, 1995. *FEBS Letts.* 377:295-300. The binding of 53BP2 to PP1 inhibits the latter protein's ability to dephosphorylate the p53 protein. Although PP1-mediated phosphorylation of p53 at multiple sites has been demonstrated to affect the transcriptional activation/inhibition of the protein, this interaction is highly complex and difficult to elucidate in a quantitative, predictable manner. See e.g., Hecker, *et al.*, 1996. *Oncogene* 12:953-961.

Accordingly, 53BP2 serves an important role in the control of cell cycle progression, transcriptional regulation, cellular apoptosis and differentiation, intracellular signal transduction, and tumorigenesis.

(2) β-TUBULIN

The human β-tubulin protein [GenBank Acc. No. X79535] has been shown to exist in at least six different isoforms which are expressed from separate genes in a tissue-specific manner. See e.g., Ranganathan, *et al.*, 1997. *Prostate* 30: 263-268. The tubulin proteins are critical to the enzymatically-mediated conversion of ATP hydrolysis to mechanical/molecular movement along microtubules. The carboxyl-terminus of β-tubulin (specifically the last 12 amino acid residues) interacts with kinesin motors to modulate microtubule polymerization, dynamics, and drug sensitivity. See e.g., Tucker & Goldstein, 1997. *J. Biol. Chem.* 272:9481—9488. Interestingly, these aforementioned functions of β-tubulin may have pathophysiological significance, as type IV β-tubulin is highly expressed in adenocarcinomas of the prostate and type II tubulin expression is up-regulated in adenocarcinomas which become malignant. See e.g., Ranganathan, *et al.*, 1997. *Prostate* 30: 263-268. Colchicine, which specifically interacts with β-tubulins to

arrest cellular outgrowth, ahs been shown to function as an effective anti-tumor agent. See e.g., Banerjee, 1997. *Biochem. Biophys. Res. Commun.* 231:698-700. Further, microtubules (possibly through β-tubulin binding to proteins which possess *Src* homology 2 (SH2) domains) play important roles in the assembly of signaling molecular complexes involved in cellular transformation processes. See e.g., Itoh, *et al.*, 1996. *J. Biol. Chem.* 217:27931-27935.

Therefore, in summary, β-tubulin serves a role in tumorigenesis and tumor progression, cell structure and intracellular protein transport, cell differentiation, and intracellular signaling

(3) THE p62 PROTEIN

The human p62 protein [GenBank Acc. No. M88108] is a 62 kD tyrosine-specific kinase which displays significant homology to the hnRNP protein GRP33. See e.g., Wong, *et al.*, 1992. *Cell* 69:551-558. The p62 protein has been demonstrated to associate with the $p21^{waf}$ GTPase-activating protein (GAP), wherein the binding of p62 is dependent upon its phosphorylation state, and occurs via the SH2 domains within GAP. See e.g., Wong, *et al.*, 1992. *Cell* 69:551-558. In addition, the p62 protein also has been shown to associate with the 5H3 domains possessed by the family of *Src* tyrosine kinases. As p62 possesses the ability to simultaneously interact with multiple proteins, via a plurality of SH3 binding domains, the p62 protein serves to physically link *Src* kinase activity with downstream effectors including, but not limited to, GRB2 and phospholipase C γ-l. See e.g., Richard, *et al.*, 1995. *Mol. Cell. Biol.* 15:186-197.

Furthermore, in its dephosphorylated state, p62 has been demonstrated to specifically interact with RNA via a KH domain. See e.g., Wang, *et al.*, 1995. *J. Biol. Chem.* 270:2010-2013. Phosphorylation severely impairs p62 binding to RNA, thus suggesting that the binding of the p62 protein to RNA is regulated by a phosphorylation/dephosphorylation mechanism *in vivo*. p62 is known to specifically interact with the ubiquitin protein, via an 80 amino acid residue domain at its carboxyl-terminus (see *e.g.*, Vadlamudi, *et al.*, 1996. *J. Biol. Chem.* 271:20235-0237), thus implicating p62 in ubiquitin-mediated proteolysis. The p62 protein also specifically interacts with CSK (a cytosolic tyrosine kinase which negatively-regulates the *Src* family of tyrosine kinases). It has been hypothesized that the binding of p62 to CSK mediates the interaction (*i.e.*, docking) of proteins (*e.g.*, CSK and GAP) to cytoskeletal and membranal regions upon c-*Src* activation. See e.g., Neet & Hunter, 1995. *Mol. Cell. Biol.* 15:4908-4920. Intracellular levels of phosphorylated p62 (as detected by Western blotting), are markedly increased in v-*abl* transformed lymphoblasts (a cell model of leukemia) which subsequently

reach advanced stages of feeder-layer-independent agar growth. See e.g., Clark & Liang, 1995. *Leukemia* 9:165-174.

Hence, in summary, the p62 protein serves an important role in the processes of cell transformation and tumor progression, intracellular signaling and cellular activation by c-*Src*, ubiquitin-mediated proteolysis, and mRNA binding and metabolism.

(4) THE hnRNP G PROTEIN

The human hnRNP G protein [GenBank Acc. No. Z23064] is an RNA-binding protein whose homolog (p43) was originally identified as an auto-antigenic nuclear protein in canine models a systemic lupus erythematosus (SLE)-like syndrome. See e.g., Soulard, *et al.*, 1993. *Nuc. Acids Res.* 21:4210-4217. The hnRNP G protein is a glycosylated component of heterogeneous nuclear ribonucleoprotein (RNP) complexes which contains an RNA-specific binding domain at its amino-terminus and a carboxyl-terminal domain which is rich in serine, arginine, and glycine amino acid residues. See e.g., Soulard, *et al.*, 1993. *Nuc. Acids Res.* 21: 4210-4217. Likely biological functions for the hnRNP G protein include regulation of cell division, translational, and transcription. Additionally, hnRNP G may also function in various autoimmune diseases, such as SLE and rheumatoid arthritis.

The novel 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins of the present invention are encoded, in part, by a nucleotide sequence identified within the GenBank Database as EST R72810 [GenBank Acc. No. 157775] initially obtained from the Soares (human) breast library 2NbHBst. Over a span of 54 nucleotides, the EST R72810 sequence displays a 74% homology to the Simian immunodeficiency virus (SIV) ptS gene [GenBank Accession No. U05129], but otherwise displays no significant homology to other characterized proteins.

SUMMARY OF THE INVENTION

The present invention discloses both compositions and methods of production for protein complexes of the 53BP2 protein with various other proteins which interact with 53BP2. It should be noted that proteins which interact with the 53BP2 protein are hereinafter designated as 53BP2 interacting-protein "53BP2-IP" and complexes of the 53BP2 protein with a given 53BP2-IP are hereinafter designated as "53BP2•53BP2-IP."

More specifically, the present invention relates to complexes of the 53BP2 protein (and derivatives, fragments and analogs thereof) with: (*i*) β-tubulin, (*ii*) p62, (*iii*) hnRNP G, (*iv*) 53BP2:IP-1, (*v*) 53BP2:IP-2 and (vi) 53BP2:IP-3. (and their derivatives, analogs and fragments). The present invention further provides methodologies for the screening of proteins which interact with the 53BP2 protein (or derivatives, fragments or analogs thereof), wherein the preferable screening methodology is a yeast two hybrid assay system, or a modification thereof.

The present invention further relates to the nucleotide sequences of 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 genes (*e.g.*, human 53BP2:IP-l, 53BP2:IP-2, and 53BP2:IP-3 genes and homologs of other species), as well as derivatives, fragments and analogs thereof. Also disclosed herein are nucleic acids which are complementary to the aforementioned nucleotide sequence including, but not limited to, the inverse complement. The present invention also relates to 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 (and derivatives, fragments and analogs thereof) which are biologically and functionally active (*i.e.*, are capable of displaying one or more known functional activities of a wild-type 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 proteins). Such functional activities include, but are not limited to: (*i*) the ability to bind with, or compete for binding with, the 53BP2 protein, (*ii*) antigenicity (*i.e.*, the ability to bind with, or compete with, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 for binding) to an anti-53BP2:IP-l, anti-53BP2:IP-2 or anti-53BP2:IP-3 antibody, respectively) and/or (*iii*) immunogenicity (*i.e.*, the ability to generate an antibody which binds 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3, respectively).

Methods of production of the 53BP2•53BP2-IP complexes and of 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 proteins, as well as derivatives, fragments and analogs of the complexes and proteins (*e.g.*, by recombinant means), are also disclosed by the present herein. Pharmaceutical compositions utilizing the 53BP2 protein and the aforementioned 53BP2•53BP2-IP complexes are also disclosed. The present invention further provides methodologies for the modulation (*i.e.*, the inhibition or enhancement) of the activity of 53BP2•53BP2-IP complexes, more specifically modulation of the 53BP2•β-tubulin, 53BP2•p62, 53BP2•hnRNP G, 53BP2•53BP2:IP-1, 53BP2•53BP2:IP-2 or 53BP2•53BP2:IP-3 complexes. The various protein components of these complexes have been implicated in cellular functions which include, but are not limited to: control of cell cycle progression, cellular differentiation and apoptosis, tumorigenesis and tumor progression, regulation of transcription and translation, control of intracellular signal transduction (*e.g.*, c-*Src* signaling), control of ubiquitin-mediated protein degradation, and processing involving mRNA binding and stability. Accordingly, the present

invention provides methodologies for the screening 53BP2•53BP2-IP complexes (and derivatives, fragments and analogs thereof) for the ability to alter cell functions, particularly those cell functions in which the 53BP2 protein and/or a 53BP2-IP have been implicated. These cellular function include, but are not limited to: (*i*) cell proliferation, differentiation and apoptosis; (*ii*) tumorigenesis and cell transformation; (*iii*) intracellular signal transduction; (*iv*) gene expression (*v*) ubiquitin-mediated protein degradation and (*vi*) mRNA stability.

The present invention also discloses therapeutic and prophylactic, as well as diagnostic, prognostic and screening methodologies and compositions which are based upon 53BP2•53BP2-IP complexes (and the nucleic acids encoding the individual proteins which participate in the complexes) as well as 53BP2:IP-l; 53BP2:IP-2 and 53BP2:IP-3 proteins and nucleic acids. Therapeutic compounds of the present invention include, but are not limited to: (*i*) 53BP2•53BP2-IP complexes and complexes where one or both members of the complex is a derivative. fragment or analog of the 53BP2 protein, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2-IP-3 proteins (and derivatives, fragments and analogs thereof); (*ii*) antibodies specific for the aforementioned proteins; (*iii*) nucleic acids encoding the aforementioned proteins and (*iv*) antisense nucleic acids to the nucleotide sequences encoding the complex components and 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 antisense nucleic acids. Diagnostic, prognostic and screening kits are also provided herein.

The present invention also discloses animal models and methodologies for the screening of modulators (*i.e.*, agonists, antagonists and inhibitors) of the activity of 53BP2•53BP2-IP complexes and 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 proteins. Methodologies for the identification of molecules which inhibit or, alternatively, which increase the formation of 53BP2•53BP2-IP complexes are also provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: The nucleotide sequence of 53BP2 [GenBank Acc. No. U58334; SEQ ID NO: 1] and its associated, inferred amino acid sequence [SEQ ID NO:2]. The amino-terminal start site of the sequence (utilized as bait in the assays described in Section 6, *infra*) is indicated by the arrow labeled "A".

Figure 2: The nucleotide sequence [SEQ ID NO:3] and associated, inferred amino acid sequence [SEQ ID NO:4] of β-tubulin (GenBank Accession No. X7953 5). Prey sequence A begins at nucleotide 820 and amino acid residue 253 which are denoted by the arrow labeled "A". Prey sequence B begins at nucleotide 895 and amino acid residue 278 which are denoted by the arrow labeled "B."

Figure 3: The nucleotide sequence [SEQ ID NO. 5] and associated, inferred amino acid sequence [SEQ ID NO. 6] of the p62 protein (GenBank Acc. No. M88108). The amino-terminal start site of the prey sequence (utilized as bait in the assays described in Section 6, *infra*) is indicated by the arrow labeled "A."

Figure 4: The nucleotide sequence [SEQ ID NO:7] and associated, inferred amino acid sequence [SEQ ID NO:8] of the hnRNP G protein. The amino-terminal start site of the prey sequence (utilized as bait in the assays described in Section 6, *infra*) is indicated by the arrow labeled "A."

Figure 5: The nucleotide sequence of EST R72810 [SEQ ID NO:9]. The entire sequence was utilized as a prey sequence in the assays described in Section 6, *infra*.

Figure 6: A schematic diagram of the portions of the 53BP2 protein, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 (*i.e.*, the proteins potentially encoded, at least in part, by the extended EST R72810 sequence) which interact to from a 53BP2•53BP2:IP complex in the yeast two hybrid assay system. The sequences of the 53BP2 protein, β-tubulin, p62, hnRNP G and EST R78210 proteins are depicted as bars positioned at the starting and ending amino acid residues (as depicted for each protein in Figures 1-4, 9 and 10A—F (SEQ ID NOS: 2, 4, 6, 8, 11, 12 and 13, respectively). The portions of each sequence either used as bait (in the case of 53BP2) or identified in the assay ("prey sequence"). Similarly, the sequences of the β-tubulin, p62, hnRNP G and EST R78210 encoded proteins are blackened, and the first amino acid residue number of the bait or prey sequence, as the case may be, is indicated above each bar. Additionally, for β-tubulin, the amino-terminal portion of a second, longer interacting sequence is indicated by a horizontal line (with the first amino acid of this extension indicated above the bar). For 53BP2:IP-1 and 53BP2:IP-3, the first amino acid is denoted by ">1" as the actual

amino-terminus of the protein is predicted to extend beyond the 5'-terminus of the assembled nucleotide sequence.

Figure 7: A matrix of results of yeast two hybrid system assays. The yeast expressing hybrids of the bait proteins Bl, MDM2 and 53BP2 are indicated in the rows designated Bl, MDM2 and 53BP2 mated with yeast cells expressing hybrids of the prey proteins P1, P2, PP1α, p62 and β-tubulin ("β-tub."), as indicated by the rows designated as P1, P2, PP1α, p62 and β-tub, are depicted. A positive interaction for a bait and prey proteins is indicated as "+" in the box forming the intersection between the particular bait and prey proteins; whereas a lack of interaction is designated by an empty box. Boxes labeled A, B, C and D indicate the results of matings of yeast expressing Bl and PP1α, 53BP2 and PPW, 53BP2 and p62, and 53BP2 and β-tubulin, respectively.

Figure 8: An illustration of the general procedure utilized to assemble the longest possible contiguous nucleic acid sequence from a particular EST sequence. The starting EST nucleic acid sequence (denoted as the line labeled as "B") is analyzed with the NCBI. "BLAST" Program and compared to all sequences within the "nr" database. Sequences which demonstrated homology ≥ 95% (at the nucleic acid level) over their termini of at least 30 bases were utilized if the said alignment resulted in a 5'-extension (Sequence A) or 3'-extension (Sequence C) of the starting EST sequence.

Figure 9: The nucleotide sequence of EST R72810 and 25 contiguous EST sequences [SEQ ID NO:10] is depicted. The original EST R72810 sequence is shown in bold lettering. A 5'-terminus extension was achieved with EST C17385, and is denoted by the underline sequence. Similarly, 3'-terminus extensions were made, first from EST AA464793 (denoted by boxed lettering) and secondly with EST AA479761 (denoted by bold, italic lettering). The 5'-terminus of the prey interacting sequence is denoted by "A"; whereas the 3'-terminus of the sequence itself is denoted by a starred arrow.

Figures 10A-F: The predicted open reading frames (ORFs) and translation of the open reading frames, in all three possible reading frames, of the nucleotide sequence of SEQ ID NO:10 (A and B). The carboxyl-terminus of 53BP2:IP-3 [SEQ ID NO:13], which is encoded by Translation Frame +1 at the 5'-terminus of the assembled nucleotide sequence, is depicted

graphically in Panel A. Panel B represents the nucleotide and associated, inferred amino acid sequence of the carboxyl-terminus of 53BP2:IP-3. 53BP2:IP-2 [SEQ ID NO:12], which is encoded by Translation Frame +2 from nucleotides 44-652, is depicted in Panel C. Panel D represents the nucleotide and associated, inferred amino acid sequence of 53BP2:IP-2. 53BP2:IP-1 [SEQ ID NO:11], which is encoded in Translation Frame +2 from nucleotides 44-652, is depicted in Panel E. Panel F represents the nucleotide and associated, inferred amino acid sequence of 53BP2:IP-1.

DETAILED DESCRIPTION OF THE INVENTION

The present invention disclosed herein relates to the identification of proteins which interact with the 53BP2 protein (the 53BP2-interacting proteins are hereinafter referred to as "53BP2-IPs") by the utilization of an improved, modified form of the yeast two hybrid system. β-tubulin, p62, hnRNP G, and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 were demonstrated to form complexes under physiological conditions with the 53BP2 protein (the complexes of the 53BP2 protein with a 53BP2-IP are hereinafter referred to as "53BP2•53BP2-IP" complexes"). These 53BP2•53BP2-IP complexes, by virtue of the interaction, are implicated in the modulation of the functional activities of the 53BP2 protein and its associated binding partners (*i.e.*, 53BP2-IPs). Such functional activities include, but are not limited to, cell cycle control, transcriptional regulation, cellular apoptosis and differentiation, intracellular signal transduction, tumorigenesis and tumor progression, protein transport and cell structure, cell differentiation, cellular activation by c-*Src*, ubiquitin-mediated proteolysis, mRNA binding and metabolism, translational regulation, and autoimmune disease.

The present invention also relates to methodologies for the screening of proteins which interact with the 53BP2 protein. In a specific embodiment, the present invention discloses 53BP2 complexes, in particular complexes of the 53BP2 protein with one or more of the following proteins: β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3. Another specific embodiment of the present invention relates to complexes of the 53BP2 protein (or derivatives, fragments or analogs thereof) with β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 (or derivatives, fragments and analogs thereof. In another specific embodiment, these aforementioned complexes bind an anti-53BP2•53BP2-IP complex antibody. In yet another specific embodiment of the present invention, complexes of the 53BP2 protein with a protein which is not protein phosphatase 1α (PP 1α) or p53 are disclosed.

The present invention additionally provides methodologies for the production and isolation of the 53BP2•53BP2-IP complexes. In a specific embodiment, the present invention discloses methodlogies for the recombinant expression of both the 53BP2 protein and its binding partner, 53BP2-IP (or fragments, derivatives or homologs of one or both members of the complex); wherein either both binding partners are under the control of a single heterologous promoter (*i.e.*, a promoter not naturally associated with the native gene encoding the particular complex component) or where each is under the control of a separate heterologous promoter. In yet another embodiment, the present invention provides the nucleotide sequences of 53BP2:IP-l, 53BP2-IP-2 and 53BP2-IP-3, as well as the associated, inferred amino acid sequences of their respective encoded proteins. The present invention further relates to 53BP2:IP-l, 53BP2-IP-2 and 53BP2-IP-3 proteins (or derivatives, fragments, analogs and homologs thereof) as well as nucleic acids encoding the 53BP2:IP-l, 53BP2:IP-2 and 53BP2-IP-3 proteins (or, derivatives, fragments. analogs and homologs thereof). The present invention also provides 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins and the nucleic acid sequences encoding these proteins derived from many different species, preferably from vertebrates, and more preferably from mammals. In the most preferred embodiment, the 53BP2:IP-l, 53BP2-IP-2 and 53BP2:IP-3 proteins and genes are of human origin. Production of the aforementioned proteins and nucleic acid sequences by recombinant methodologies is provided herein.

The present invention further relates to 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 proteins (and derivatives and analogs thereof) which are functionally active (*i.e.*, are capable of displaying one or more of the known, functional activities associated with the wild-type 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3. Such functional activities include, but are not limited to: (*i*) the ability to form a complex with the 53BP2 protein; (*ii*) antigenicity (*i.e.*. the ability to bind, or compete with, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 for binding to an anti-53BP2:IP-1, anti-53BP2-IP-2 or anti-53BP2:IP-3 antibody) and (*iii*) immunogenicity (*i.e.*, the ability to generate an antibody which binds to 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3).

Methods of diagnosis, prognosis, and screening for diseases and disorders which are associated with aberrant levels of 53BP2•53BP2-IP complexes or of 53BP2:IP-l, 53BP2-IP-2 or 53BP2-IP-3, are also provided herein. The present invention also discloses methodologies for the therapeutic or prophylactic treatment of diseases or disorders which are associated with aberrant levels of 53BP2•53BP2-IP complexes or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 or, similarly, aberrant levels of the activity of one or more of the components of the complex. Treatment may be facilitated by, but is not limited to, the administration of: (*i*) 53BP2•53BP2-IP

complexes, 53BP2:IP-1, 53BP2:IP-2 53BP2:IP-3, (*ii*) modulators of 53BP2•53BP2-IP complex formation or activity (*e.g.*, antibodies which bind the 53BP2•53BP2-IP complex, the non-complexed 53BP2 protein or 53BP2-IP, or a derivative or fragment thereof-which preferably possesses the region containing the portion of 53BP2 or the 53BP2-IP that is directly involved in complex formation); (*iii*) mutants of the 53BP2 protein or the 53BP2-IP which increase or decrease binding affinity; (*iv*) small molecule inhibitors/enhancers of complex formation and (*v*) antibodies which either stabilize or neutralize the complex. Methodologies for assaying 53BP2•53BP2-IP complexes, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 for biological activity as therapeutics or diagnostics, as well as methodologies for the screening of 53BP2•53BP2-IP complexes, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 modulators (*i.e.*, inhibitors, agonists and antagonists) are also disclosed herein.

It should be noted that, for clarity of disclosure, and not by way of limitation, the detailed description of the present invention is divided into the subsections which follow, *infra*.

(1) 53BP2•IP COMPLEXES AND 53BP2:IP-1, 53BP2:IP-2 AND 53BP2:IP-3 PROTEINS

The present invention relates to 53BP2•53BP2-IP complexes, and, in specific embodiments, complexes of the 53BP2 protein and β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3. In a preferred embodiment, the 53BP2 protein is complexed with a protein which *is not* a PP1α or a p53 protein. In another preferred embodiment, the 53BP2•53BP2-IP complexes are complexes of human proteins. The present invention also relates to complexes of the 53BP2 proteins and 53BP2-IP wherein one or both members of the complex are fragments, derivatives or analogs of the wild-type 53BP2 or 53BP2-IP protein. Preferably, the 53BP2•53BP2-IP complexes possess one or both members which are functionally-active fragments, derivatives or analogs of the wild-type protein(s). The term "functionally-active 53BP2•53BP2-IP complex," as utilized herein, refers to those complexes which display one or more of the known functional attributes of a complex of full-length 53BP2 protein with a full-length 53BP2-IP (*e.g.*, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3). These functional activities include, but are not limited to: (*i*) cell cycle control, (*ii*) modulation of cell apoptosis and differentiation; (*iii*) control of transcriptional and translational regulation; (*iv*) effects on intracellular signal transduction, protein transport, and c-Src activation; (*v*) effects on tumorigenesis and tumor progression; (*iv*) ubiquitin-mediated proteolysis; (*vi*) effects on mRNA binding and metabolism; (*vii*) binding to an anti-

53BP2•53BP2-IP complex antibody and (*viii*) various other biological activities well-known within the art.

For example, the derivatives or analogs of the 53BP2 protein which possess the desired immunogenicity or antigenicity may be utilized in immunoassays, as immunogens for antibody production, for inhibition of 53BP2•53BP2-IP complex activity, and the like. Similarly, derivatives or analogs which retain, or alternatively lack or inhibit, a specific property of interest (*e.g.*, participation in the formation of a 53BP2•53BP2-IP complex) may be used as inducers or inhibitors, respectively, of such a property and its physiological correlates. Such derivatives or analogs of 53BP2•53BP2-IP complexes may be screened for the desired biological activity by any of the procedures well-known within the art.

In specific embodiments of the present invention, 53BP2•53BP2-IP complexes comprising fragments of one or both members of the complex are disclosed. In a preferred embodiment, fragments of various 53BP2-IPs which have been shown to form complexes with the 53BP2 protein by use of the yeast two hybrid assay methodology are disclosed. These aforementioned fragments may consist of, but are not limited to: (*i*) the carboxyl-terminal domain of the 53BP2 protein (amino acid residues 704-1005) as depicted in Figure 1 [SEQ ID NO:2]; (*ii*) amino acid residues 253-445 or 278-445 of β-tubulin, as depicted in Figure 2 [SEQ ID NO:4]; (*iii*) amino acid residues 275-443 of p62, as depicted in Figure 3 [SEQ. ID NO:6]; (*iv*) amino acid residues 88-439 of hnRNP G, as depicted in Figure 4 [SEQ ID NO:8]; (*v*) amino acid residues 8-173 of 53BP2:IP-1, as depicted in Figure 10F [SEQ ID NO:11]; (*vi*) amino acid residues 1-70 of 53BP2:IP-2, as depicted in Figure 10D [SEQ ID NO:12] and (*vii*) and amino acid residues 8-33 of 53BP2:IP-3. as depicted in Figure 10B [SEQ ID NO:13].

The nucleic acid sequences (*i.e.*, genes) encoding the aforementioned proteins, as well as derivatives, fragments and analogs thereof, are also disclosed by the present invention. The nucleotide and associated amino acid sequences of the human 53BP2 protein (GenBank Acc. No. U58334); β-tubulin (GenBank Acc. No. X79535); p62 (GenBank Acc. No. M88108) and hnRNP G (GenBank Acc. No. Z23064) have been previously elucidated, and are provided in Figures 1-4 and SEQ ID NOS:1, 3, 5 and 7, respectively. The nucleic acid sequences encoding the 53BP2 protein, β-tubulin, p62 or hnRNP G may be obtained by any method known within the art (*e.g.*, by polymerase chain reaction-mediated amplification using synthetic primers hybridizable to the 3'- and 5'-termini of the sequence of interest and/or by cloning from a cDNA or genomic library using an oligonucleotide which is specific for the gene sequence of interest). Homologs (*i.e.*, nucleic acids encoding the 53BP2 protein, β-tubulin, p62, and hnRNP G of

species other than human) or other related sequences (*e.g.*, paralogs) may be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe utilizing methodologies for nucleic acid hybridization and cloning which are well-known within the art.

The 53BP2, β-tubulin, p62, hnRNP G, and 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 proteins (either alone or in a complex) may be obtained by methods well-known within the art for protein purification and recombinant protein expression. For recombinant expression of one or more of the proteins, the nucleic acid containing all or a portion of the nucleotide sequence encoding the protein of interest may be inserted into an appropriate expression vector (*i.e.*, a vector which contains the requisite elements for the transcription and translation of the inserted protein coding sequence). These necessary transcriptional and translational signals may also be supplied by the native promoter for the 53BP2 gene or any of the genes encoding the 53BP2-IPs and/or their flanking regions. A variety of host-vector systems may be utilized to express the protein coding sequence including, but not limited to: (*i*) mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, and the like); (*ii*) insect cell systems infected with virus (*e.g.*, baculovirus)or (*iii*) microorganisms such as yeast containing yeast vectors or bacteria transformed with bacteriophage, plasmid or cosmid DNA. Accordingly, depending upon the specific host-vector system utilized, any one of a number of suitable transcription and translation elements may be used in the practice of the present invention.

In a preferred embodiment, the 53BP2•53BP2-IP complexes are obtained by expression of the entire 53BP2 and 53BP2-IP coding sequences within the same cell, either under the control of the same promoter or two separate promoters. In another preferred embodiment, a derivative, fragment or homolog of the 53BP2 protein and/or a derivative, fragment or homolog of a 53BP2-IP are recombinantly expressed. Preferably the derivative, fragment or homolog of the 53BP2 protein and/or the 53BP2-IP possess the ability to form a complex with a binding partner identified by a binding assay (*e.g.*, the modified yeast two hybrid system). Any of the methods well-known within the art for the insertion of DNA fragments into a vector may be utilized to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequences encoding the 53BP2 protein and a 53BP2-IP (*e.g.*, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3), or derivatives, fragments or homologs thereof, may be regulated by a second nucleic acid sequence

such that the genes or fragments thereof are expressed in a host transformed with the recombinant DNA molecule(s).

In a specific embodiment, the promoter is not native to the genes for the 53BP2 protein or the 53BP2-IP. Promoters which may be utilized include, but are not limited to: (*i*) the SV40 early promoter (see *e.g.*, Bernoist & Chambon, 1981. *Nature* 290:304-310); (*ii*) the promoter contained in the 3'-terminus long terminal repeat of Rous sarcoma virus (see *e.g.*, Yamamoto, *et al.*, 1980. *Cell* 22:787-797); (*iii*) the Herpesvirus thymidine kinase promoter (see *e.g.*, Wagner, *et al.*, 1981. *Proc. Natl. Acad. Sci. USA* 78:1441-1445); (*iv*) the regulatory sequences of the metallothionein gene (see *e.g.*, Brinster, *et al.*, 1982. *Nature* 296:39-42); (*v*) prokaryotic expression vectors such as the β-lactamase promoter (see *e.g.*, Villa-Kamaroff, *et al.*, 1978. *Proc. Natl. Acad. Sci. USA* 75:3727-3731) or (*vi*) the *tac* promoter (see *e.g.*, DeBoer, *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80:21-25. In addition, animal transcriptional control regions which exhibit tissue specificity and have been utilized in transgenic animals may also be utilized. These transcriptional control regions include, but are not limited to: (*i*) the elastase I gene control region which is active in pancreatic acinar cells (see *e.g.*, Swift, *et al.*, 1984. *Cell* 38:639-646; (*ii*) the insulin gene control region which is active in pancreatic β-cells (see *e.g.*, Hanahan, *et al.*, 1985. *Nature* 315:115-122); (*iii*) the immunoglobulin gene control region which is active in lymphoid cells (see *e.g.*, Alexander, *et al.*, 1987. *Mol. Cell Biol.* 7:1436-1444); (*iv*) the mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (see *e.g.*, Leder, *et al.*, 1986. *Cell* 45:485-495); (*v*) the α-fetoprotein gene control region which is active in liver (see *e.g.*, Krumlauf, *et al.*, 1985. *Mol. Cell. Biol.* 5:1639-1648); (*vi*) the β-globin gene control region which is active in myeloid cells (see *e.g.*, Kollias, *et al.*, 1986. *Cell* 46:89-94) and (*vii*) the myosin light chain-2 gene control region which is active in skeletal muscle (see *e.g.*, Shani, 1985. *Nature* 314:283-286). In a specific embodiment of the present invention, a vector is utilized which comprises: (*i*) a promoter operably-linked to nucleic acid sequences encoding the 53BP2 protein and/or a 53BP2-IP (*e.g.*, β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3), or a fragment, derivative or homolog thereof; (*ii*) one or more origins of replication and optionally, (*iii*) one or more selectable markers (*e.g.*, an antibiotic resistance gene). In a preferred embodiment of the present invention, a vector is utilized which comprises a promoter operably-linked to nucleic acid sequences encoding both the 53BP2 and a 53BP2-IP, one or more origins of replication, and one or more selectable markers. For example, in a specific embodiment, an expression vector containing the coding sequences, or portions thereof, of the 53BP2 protein and a 53BP2-IP, either together or separately, is produced by subcloning the

aforementioned gene sequences into the *EcoRI* restriction site of each of the three pGEX vectors (glutathione 5-transferase expression vectors; see *e.g.*, Smith & Johnson, 1988. *Gene* 7:31-40), thus allowing the expression of products in the correct reading frame.

Expression vectors containing the sequences of interest may be identified by three general approaches: (*i*) nucleic acid hybridization, (*ii*) presence or absence of "marker" gene function and (*ii*) expression of the inserted sequences. In the first approach, the 53BP2 protein, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2, 53BP2:IP-3, or other 53BP2-IP sequences may be detected by nucleic acid hybridization utilizing probes comprising sequences which are homologous and complementary to the inserted sequences of interest. In the second approach, the recombinant vector/host system may be identified and selected based upon the presence or absence of certain "marker" functions (*e.g.*, binding to an anti-53BP2, anti-53BP2-IP or anti-53BP2•53BP2-IP complex antibody, resistance to antibiotics, occlusion body formation in baculovirus, and the like) caused by the insertion of the sequence(s) of interest into the vector. For example, if the 53BP2 or a 53BP2-IP gene (or portion thereof) is inserted within the marker gene sequence of the vector, recombinants containing the 53BP2 or 53BP2-IP fragment will be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors may be identified by assay for the 53BP2 protein, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2, 53BP2:IP-3, or other 53BP2-IP products which may be expressed by the recombinant vector. Such assays can be based, for example, on the physical or functional properties of the interacting species in *in vitro* assay systems (*e.g.*, formation of a 53BP2•53BP2-IP complex, immunoreactivity to antibodies specific for the protein and the like).

Once a recombinant 53BP2, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2, or 53BP2:IP-3, or other 53BP2-IP molecule is identified and the complexes or individual proteins subsequently isolated, various methodologies which are well-known within the art may be utilized to "amplify" them. For example, in one embodiment of the present invention, once a suitable host system and growth conditions have been established, recombinant expression vectors are then propagated and amplified in quantity. As previously described, the expression vectors or derivatives which may be used include, but are not limited to: human or animal viruses such as vaccinia virus or adenovirus, insect viruses such as baculovirus, yeast vectors, bacteriophage vectors such as lambda phage, and plasmid and cosmid vectors. In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies or processes the expressed proteins in the specific fashion desired. The use of certain promoters allows the expression of the desired sequences to be elevated in the presence of certain inducers.

Accordingly, the expression of the genetically-engineered 53BP2 and/or 53BP2-IP may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, phosphorylation, and the like) of proteins. Appropriate cell lines or host systems may be selected to ensure the desired modification and processing of the foreign protein is achieved. For example, expression in a bacterial system can be used to produce an non-glycosylated core protein, while expression within mammalian cells ensures "native" glycosylation of a heterologous protein.

In other specific embodiments, the 53BP2 and/or 53BP2-IPs (or derivatives, fragments or homologs thereof) may be expressed as fusion or chimeric protein products comprising the protein (or derivative, fragment or homolog thereof) joined via a peptide bond to a heterologous protein sequence of a different protein. These aforementioned chimeric products may be generated by the ligation of the appropriate nucleic acid sequences encoding the desired amino acids to one another (in the proper "reading frame") and expressing the chimeric products in a suitable host by methodologies which are well-known with the relevant art. Alternatively, chimeric product may be produced by protein synthetic techniques (*e.g.*, by use of a peptide synthesizer).

Chimeric genes comprising portions of the 53BP2 protein and/or a 53BP2-IP, or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 which are fused to any heterologous protein-encoding sequences may be constructed in the practice of the present invention. A specific embodiment of the present invention relates to a chimeric protein comprising a fragment of the 53BP2 protein and/or a 53BP2-IP, or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 of at least six amino acid residues. In another specific embodiment of the present invention, fusion proteins are provided which contain the interacting domains of the 53BP2 protein and a 53BP2-IP (*e.g.*, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3) and, optionally, a peptide linker between the two domains, where such a linker promotes the interaction of the 53BP2 and 53BP2-IP binding domains. These aforementioned fusion proteins may be particularly useful where the stability of the interaction is desirable (due to the formation of the complex as an intramolecular reaction), for example in production of antibodies specific to the 53BP2•53BP2-IP complex.

In yet another specific embodiment of the present invention, derivatives of the 53BP2 protein and/or 53BP2-IP or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 may be produced by the alteration of their sequences by substitutions, additions or deletions which provide for functionally-equivalent molecules. As a function of the degeneracy of nucleotide coding

sequences, other DNA sequences which encode substantially the same amino acid sequence as a 53BP2 or 53BP2-IP or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 gene may be utilized in the practice of the present invention. These nucleotide sequences include, but are not limited to, the sequences comprising all or portions of the 53BP2 protein, β-tubulin, p62, hnRNP C, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3, or other 53BP2-IP genes which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a "silent" substitution. Similarly, the 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 derivatives of the present invention include, but are not limited to, those derivatives containing, as a primary amino acid sequence, all or part of the amino acid sequence of 53BP2 or a 53BP2-IP or 53BP2:IP-1, 53BP2-IP-2 or 53BP2-IP-3, including altered sequences in which functionally-equivalent amino acid residues are substituted for residues within the sequence resulting in a "silent" substitution. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent. Substitutes for an amino acid within the sequence may be selected from other members of the class (*i.e.*, hydrophobic or hydrophilic) to which the amino acid residue belongs.

A specific embodiment of the present invention discloses the nucleic acid sequences which encode the proteins and proteins consisting of or comprising a fragment of the 53BP2 protein or a 53BP2-IP or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 consisting of at least 6 (continuous) amino acid residues. In other specific embodiments of the present invention, the fragment consists of at least 10, 20, 30, 40, or 50 amino acid residues of the 53BP2 protein, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3. In yet another specific embodiments, such fragments are not larger than 35, 100 or 200 amino acid residues. Derivatives or analogs of the 53BP2 protein and 53BP2-IPs or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 include, but are not limited to, molecules comprising regions which are substantially homologous to the 53BP2 protein, 53BP2-IPs, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 in various embodiments, and demonstrated homologies ranging from 35-95%, with preferable homology of 85%, more preferable homology of 90% and most preferable homology of 95%, to an amino acid sequence of identical size or when these aforementioned derivatives or analogs are compared to an aligned sequence in which the alignment is performed by a computer homology program known in the art or whose encoding nucleic acid is capable of hybridizing to a sequence encoding the 53BP2 protein, a 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3, under stringent, moderately

stringent, or non-stringent hybridization conditions. Homology algorithms utilized in the present invention include, but are not limited to, the BLASTN/BLASTX, BLASTP and FASTA.

The 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 derivatives and analogs of the present invention may be produced by various methods known within the art and the manipulations which result in their production may occur at the gene or protein level. The cloned 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 gene sequences may be modified by any of numerous strategies known within the art. See *e.g.*, Maniatis, T., 1990. *Molecular Cloning, A Laboratory Manual, 2d ed.* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). For example, the nucleic acid sequences of interest may be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification (if required), isolated, and ligated *in vitro*. In the production of the nucleic acid sequence encoding a derivative or analog of the 53BP2 protein or a 53BP2-IP, care is taken to ensure that the modified sequence retains the original translational reading frame, uninterrupted by translational stop signals, in the "region" where the desired biological activity is encoded.

Additionally, the 53BP2- and/or 53BP2-IP-encoding nucleic acid sequences, as well as the 53BP2:IP-1-, 53BP2:IP-2- or 53BP2:IP-3-encoding nucleic acid sequences may be mutated *in vitro* or *in vivo*, so as to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy pre-existing ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known within the art may be utilized including, but not limited to, chemical mutagenesis and *in vitro* site-directed mutagenesis (see *e.g.*, Hutchinson, *et al.*, 1978. *J. Biol. Chem.* 253:6551-6558), use of TAB® linkers (Pharmacia), and the like.

Once a recombinant cell expressing the 53BP2 protein and/or a 53BP2-IP, or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein (or a derivative, fragment or analog thereof) is identified, the individual gene product or protein complex may be isolated and analyzed. This is achieved by utilization of assays which are based upon the physical and/or functional properties of the protein or protein complex, including, but not limited to, radioactive labeling of the gene product followed by gel electrophoretic analysis, immunoassay, cross-linking to marker-labeled product, and the like. The 53BP2•53BP2-IP complexes and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins may be isolated and purified by standard methods well-known within the art (either from natural sources or recombinant host cells expressing the proteins or protein complexes). These methodologies including, but not limited to: (*i*) chromatography (*e.g.*, ion exchange, affinity, gel exclusion, reversed-phase high pressure, fast protein liquid, etc.); (*ii*) differential centrifugation;

(*iii*) differential solubility or any other standard technique utilized for the purification of proteins. Functional properties may then be evaluated using any suitable assay known within the art. Alternatively, once a 53BP2-IP (or its derivative) is identified, the associated amino acid sequence of the protein may be deduced from the nucleic acid sequence of the chimeric gene from which it was originally encoded. As a result, the protein or its derivative may then be synthesized by standard chemical methods known within the art. See *e.g.*, Hunkapiller, *et al.* 1984. *Nature* 310:105-111.

In a specific embodiment of the present invention, the 53BP2•53BP2-IP complexes and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins (whether produced by recombinant DNA techniques, chemical synthesis methods or by purification from native sources) include, but are not limited to those which possess, as a primary amino acid sequence, all or part of the amino acid sequences substantially as depicted in Figures 1-4, and 1OB, D and F [SEQ ID NOS:2, 4, 6, 8, 13, 12 and 11], respectively. Manipulations of the 53BP2 protein and/or 53BP2-IP sequences or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 sequences, may be performed at the protein level. Included within the scope of the present invention are complexes of 53BP2 or 53BP2-IP derivatives, fragments or analogs, as well as 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 derivatives, fragments and analogs, which are differentially-modified during or after translation, (*e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, and the like). Any of numerous chemical modifications may be carried out by known techniques including, but not limited to, specific chemical cleavage by cyanogen bromide. trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.

In specific embodiments, the 53BP2 protein and/or 53BP2-IP sequences may be modified to include a detectable label which may include, but are not limited to, radioactive fluorescent, chemiluminescent, colorimeteric, and enzymatic labels. In another specific embodiment, the 53BP2 protein and/or the 53BP2-IP are modified to include a heterofunctional reagent, which may be utilized to cross-link the various proteins to other members of the complex or to other 53BP2-IPs. In addition, complexes of analogs and derivatives of the 53BP2 protein and/or a 53BP2-IP, as well as analogs and derivatives of 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 may be chemically synthesized. For example, a peptide corresponding to a portion of the 53BP2 protein, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 and/or 53BP2:IP-3, which comprises the desired domain or which mediates the desired activity *in vitro* (*e.g.*, 53BP2•53BP2-IP complex formation), may be

synthesized by use of a peptide synthesizer. Furthermore, if so desired, non-classical amino acids or chemically-modified amino acid analogs may be introduced as either a substitution or addition into the 53BP2 protein, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 and/or 53BP2:IP-3 amino acid sequences. Non-classical amino acids include, but are not limited to: the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-aminobutyric acid, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionoic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine and the like. Furthermore, the incorporated amino acid residue may be D (dextrorotary) or L (levorotary) isomers.

In cases where natural products are suspected of being mutants or are isolated from new species, the amino acid sequence of the 53BP2 protein, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 isolated from the natural source, as well as those expressed *in vitro*, or from synthesized expression vectors *in vivo* or *in vitro*, may be determined from analysis of the nucleic acid sequence, or alternatively, by direct amino acid sequencing of the isolated protein. Such analysis may be performed by manual sequencing or through use of an automated amino acid sequenator. The 53BP2•53BP2-IP complexes and 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 proteins may also be analyzed by hydrophilicity analysis (see *e.g.*, Hopp & Woods, 1981. *Proc. Natl. Acad. Sci. USA* 78:3824-3828) which can be utilized to identify the hydrophobic and hydrophilic regions of the proteins and help predict their orientation in designing substrates for experimental manipulation (*e.g.*, binding experiments, antibody synthesis, and the like). In addition, secondary structural analysis may also be performed to identify regions of the 53BP2 protein, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 and/or 53BP2:IP-3, which assume specific structural motifs. See *e.g.*, Chou & Fasman, 1974. *Biochemistry* 13:222-23. It should be noted that manipulation, translation, secondary structure prediction, hydrophilicity and hydrophobicity profiles, open reading frame (ORF) prediction and plotting, and determination of sequence homologies, may be accomplished utilizing the computer software programs currently available in the art.

Other methods of structural analysis including, but not limited to: (*i*) X-ray crystallography (see *e.g.*, Engstrom, 1974. *Biochem. Exp. Biol.* 11:7-13); (*ii*) mass spectroscopy and gas chromatography (see *e.g.*, *Methods in Protein Science* 1997. (J. Wiley and Sons, New York, NY)) and (*iii*) computer modeling (see *e.g.*, Fletterick & Zoller, 1986. Computer Graphics and Molecular Modeling, In: *Current Communications in Molecular Biology* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor Press, NY)) may also be employed.

(2) IDENTIFICATION AND ISOLATION OF 53BP2:IP-1, 53BP2:IP-2 AND 53BP2:IP-3 GENES

The present invention relates to the nucleotide sequences of nucleic acids encoding 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3. In specific embodiments, the 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 nucleic acids comprise the sequence set forth in SEQ ID NO:10 (or the coding regions thereof) or nucleotide sequences encoding, in whole or in part, a 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 protein (*e.g.*, a protein comprising the sequence of SEQ ID NOS:11, 12 and 13, respectively). The present invention also provides purified nucleic acids consisting of at least 8 continuous nucleotides (*i.e.*, a sequence which is capable of undergoing hybridization) of a 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 sequence. In yet other embodiments, the nucleic acids consist of at least 25 (continuous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of a 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 sequence, or a full-length 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 coding sequence. In still another embodiment of the present invention, the nucleic acids are smaller than 35, 200 or 500 nucleotides in length. The aforementioned nucleic acids may be single- or double-stranded.

The present invention also discloses to nucleic acids which are hybridizable or complementary to the aforementioned sequences; in particular the present invention provides the inverse complement to nucleic acids hybridizable to these foregoing sequences. In specific embodiments, nucleic acids are disclosed which comprise a sequence complementary to (specifically are the inverse complement of) at least 10, 25, 50, 100, or 200 nucleotides, or the entire coding region of a 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 gene. In a specific embodiment of the present invention, a nucleic acid which is hybridizable to a 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 nucleic acids (*e.g.*, possessing sequence SEQ ID NO:10), or to a nucleic acid encoding a 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 derivative, under conditions of low stringency are provided. By way of example and not of limitation, procedures utilizing such conditions of low stringency hybridization are as follows (see *e.g.*, Shilo & Weinberg, 1981. *Proc. Natl. Acad. Sci. USA* 78:6789-6792): filters containing the nucleic acid sequences of interest were pre-hybridized for 6 hours at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA. Hybridizations were performed in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% Bovine serum albumin (BSA), 100 µg/ml salmon sperm DNA and 10% (wt/vol) dextran sulfate. In addition, 5-20 x 10^6 cpm of ^{32}P-

labeled probe was used. Filters were incubated in the hybridization mixture for 18-20 hours at 40°C and washed for 1.5 hours at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA and 0.1% SDS. The wash solution was then replaced with fresh solution and incubated an additional 1.5 hours at 60°C. Filters were blotted dry and exposed to X-ray film for autoradiography. If necessary, filters were washed for a third-time at 65-68°C, and re-exposed to film. Other conditions of low stringency hybridization which may be used are well-known in the art (*e.g.*, as employed for cross-species hybridizations).

In a second specific embodiment of the present invention, a nucleic acid, which is hybridizable to a 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 nucleic acids under conditions of moderate stringency are disclosed. By way of example, but not of limitation, the procedures using such conditions of moderate stringency hybridization are as follows: filters containing the nucleic acids of interest were pre-hybridized for 6 hours at 55°C in a solution containing 10X SSC, 5X Denhardt's solution, 0.5% SDS and 100 μg/ml denatured salmon sperm DNA. Hybridizations were performed in the same solution and 5-20 x 10^6 cpm of ^{32}P-labeled probe was used. Filters were incubated in the hybridization mixture for 18-20 hours at 55°C and washed twice for 30 minutes at 60°C in a solution containing lX SSC and 0.1% SDS. The filters were then blotted dry and exposed to X-ray film for autoradiography. Other conditions of moderate stringency which may be used are well-known within the art.

In another specific embodiment of the present invention, a nucleic acid which is hybridizable to a 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 nucleic acids under conditions of high stringency are provided. By way of example, and not of limitation, procedures using such conditions of high stringency hybridization are as follows: pre-hybridization of filters containing the nucleic acid of interest was performed for 8 hours to overnight at 65°C in buffer composed of 6X SSC. 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 500 μg/ml denatured salmon sperm DNA. Filters were hybridized for 48 hours at 65°C in pre-hybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20x 0^6 cpm of ^{32}P-labeled probe. Washing of filters was performed at 37°C for 1 hour in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This was then followed by a wash in 0.1 X SSC at 50°C for 45 minutes prior to autoradiography. Other conditions of high stringency which may be utilized in the practice of the present invention are well-known within the art.

Nucleic acids which encode derivatives and analogs of 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 proteins, as well as 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 antisense nucleic acids are additionally provided herein. Fragments of 53BP2:IP-

1, 53BP2:IP-2 or 53BP2:IP-3 nucleic acids comprising regions conserved between (with homology to) other 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 nucleic acids, of the same or different species, are also provided.

Nucleic acids species which were predicted to encode (at least in part) 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 were identified as encoding a protein or proteins which interact with the 53BP2 protein using the improved, modified version yeast two hybrid system. As disclosed in the present invention, the 5'-terminus of this identified nucleic acid (illustrated in Figure 9) possesses a nucleotide sequence which is *identical* to the nucleotide sequence of the EST sequence EST R72810.

EST sequences are part of human DNA databases (*e.g.*, the GenBank Database "dbest"). These sequences typically represent incomplete fragments of putative genes not yet ascribed to encode a known protein or RNA species. However, these aforementioned sequences generally do not encode a full-length protein because they generally: (*i*) lack a methionine codon to act as a site of translational initiation; (*ii*) lack a translational stop codon and/or (*iii*) do not contain an open reading frame (ORF) to code for a protein longer than approximately 60 amino acid residues in length (this is shorter than the smallest currently-known translated protein). The EST databases contain many overlapping sequences, thus it is generally possible to find contiguous sequences to assemble a longer sequence representative of a larger original sequence found in nature. Common *in silico* procedures which may be used to detect homologies between nucleic acid sequences in the databases which are well-known within the art include, but are not limited to, the utilization of the "BLAST" family of programs available through the National Center for Biotechnology Information (NCBI). In order to account for potential sequencing errors, silent mutations, and the like, which are inherently present in all sequence homology computer databases, the term "significant homology," as utilized herein, may be generally defined as a nucleic acid species of interest which possesses 95-100% homology over a region of 20, 25, 30, 35, 40, or greater span of nucleotide overlap. The homology detection paradigm may allow for a limited number of single or, at most, double nucleotide insertion or deletion mismatches, particularly in regions of sequences known to be difficult to sequence, (*e.g.*, very high G + C content, multiple contiguous G residues, and the like).

These *in silico* procedures allow for the "assembly" of two sequences which overlap non-identical regions spans of a common sequence. This assembled sequence, may then be utilized to identify further-related sequences by the same procedure. The 5'- and 3'-termini of the assembled sequence are extended until significant homology to sequences within available

databases cannot be detected. The assembled EST sequence are then subjected to a final search of available databases to detect homologies to known protein sequences which were not initially detected over the shorter span of the original EST sequence. The present invention discloses several EST sequences which overlap with EST R72810 at both the 5'- and 3'-termini. These aforementioned sequences are depicted in Figures 8 and 9.

The assembled EST sequence may be analyzed by the utilization of any of a number of nucleic acid analysis computer programs which are currently available within the art, to ascertain and characterize possible protein translation products of the assembled nucleic acid sequence. It should be noted that translation in all six frames were performed in order to identify possible open reading frames (ORFs), which are contiguous spans of amino acids codons which lack a stop codon. In the case where EST sequences are derived from directionally cloned libraries, only the three forward (5' to 3') translations are required because the sense (*i.e.*, coding) strand of the EST had already been previously defined. As per standard convention, the presence of ATG start codons. which define possible sites for the initiation of protein translation, were used to identify the beginning of such an open reading frame (ORF). If an ORF (which extends to the 5'-terminus of the assembled nucleic acid sequence) was found to be longer than 60 amino acid residues in length, the assembled EST sequence was classified as a protein which potentially encodes a carboxyl-terminal region of the protein within that given reading frame (*i.e.*, a protein which is missing one or more amino-terminal amino acid residues. *In silico* analysis of the assembled EST sequence revealed three possible translation products: 53BP2:IP-l (denoting 53BP2-Interacting Protein 1); 53BP2:IP-2 (denoting 53BP2-Interacting Protein 2) and 53BP2:IP-3 (denoting 53BP2-Interacting Protein 3).

Any methodology currently available within the art may be used to obtain a full length (*i.e.*, encompassing the entire coding region) cDNA or genomic DNA clone encoding 53BP2:IP-l, 53BP2:IP-2 and/or 53BP2:IP-3. In particular, the polymerase chain reaction (PCR) may be used to amplify the sequence assembled derived from the initial EST sequences in a genomic or cDNA library. Oligonucleotide primers which hybridize to sequences at the 3'- and 5'-termini of the assembled EST sequences may be used as primers to PCR-amplify sequences from a nucleic acid sample (RNA or DNA) of interest. The nucleic acid sample is, preferably, derived from a cDNA library from an appropriate source (*e.g.*, the sample from which the initial cDNA library for the yeast two hybrid assay fusion population was derived).

PCR amplification may be performed by use of an automated thermal cycler (*e.g.*, a Perkin-Elmer Cetus® thermal cycler) and *Taq* polymerase. The nucleic acid sample being

amplified can include, but is not limited to, mRNA, cDNA or genomic DNA from any eukaryotic species. One can choose to initially synthesize several different degenerate primers, for use in the PCR amplification reactions. It is also possible to vary the stringency of hybridization conditions utilized in the annealing of the primers in the PCR reactions to facilitate the amplification of nucleic acid homologs (*e.g.*, to obtain 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 sequences from species other than humans or to obtain human sequences which possess homology to 53BP2:IP-1, 53BP2:IP-2 and/or 53BP2:IP-3) by allowing for greater or lesser degrees of nucleotide sequence homology between the known nucleotide sequence and the nucleic acid homolog of interest. Generally, for cross species hybridization, low stringency hybridization conditions are preferred. For same species hybridization, moderately stringent conditions are preferred.

Following the successful amplification of the nucleic acid containing: (*i*) all or a portion of the sequence assembled from the EST sequences or (*ii*) a nucleic acid encoding all or a portion of a 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 homolog, the nucleic acid segment may be cloned, sequenced and utilized as a probe to isolate a complete cDNA or genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra*. In this manner, the nucleotide sequences of the entire 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 genes as well as additional genes encoding proteins and analogs thereof, may also be identified.

Any eukaryotic cell potentially can serve as the nucleic acid source for the molecular cloning of the 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 genes. DNA may be obtained by standard procedures known within the art, from cloned DNA (*e.g.*, a DNA library), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA (or fragments thereof) purified from the desired cell. See *e.g.*, Glover, D.M., 1985. *DNA Cloning: A Practical Approach* (MRL Press, Ltd., Oxford, U.K). Clones which are derived from genomic DNA may contain both regulatory and intronic DNA regions, in addition to the exonic coding regions; whereas clones derived exclusively from cDNA will contain only exonic (coding) sequences.

In the molecular cloning of the gene from genomic DNA, DNA fragments are generated by cleavage with one or more restriction endonucleases (REs). Alternatively, one may use DNAse I in the presence of manganese to fragment the genomic DNA, or the DNA may be mechanically-sheared (*e.g.*, by sonication). The linear DNA fragments are then separated as a function of their molecular size by standard techniques including, but not limited to, agarose and polyacrylamide gel electrophoresis, column chromatography and the like.

Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired gene may be accomplished in a variety of ways. For example, a portion of the 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 gene (*e.g.*, a PCR amplification product obtained as described above or an oligonucleotide having a sequence of a portion of the known nucleotide sequence) or its specific RNA (or a fragment thereof) may be purified and the resulting DNA may be screened by nucleic acid hybridization to a detectably-labeled probe. See *e.g.*, Benton & Davis, 1977. *Science* 196:180-186. It is also possible to identify the appropriate fragment by: (*i*) restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map; (*ii*) DNA sequence analysis and comparison to the known nucleotide sequence of 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 and/or (*iii*) by the elucidation/ differentiation of the specific "properties" of the gene. Alternatively, the presence of the gene may be detected by assays based upon the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, may be selected on the basis of their production of a protein which, for example, possesses similar or identical electrophoretic migration, isolectric focusing behavior, proteolytic digestion maps, or antigenic properties or ability to bind the 53BP2 protein. These aforementioned properties have been ascertained in the case of 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3. Alternately, if an anti-53BP2:IP-l, anti-53BP2:IP-2 or anti-53BP2:IP-3 antibody is available, the protein may be identified by binding of labeled antibody to the putatively 53BP2:IP-l-, 53BP2:IP-2- or 53BP2:IP-3-synthesizing clones by utilization of an enzyme-linked immunosorbent assay (ELISA)-mediated procedure.

Alternatives to isolating 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or generating cDNA to the mRNA which encodes the 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 protein. For example, RNA for cDNA cloning of the 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 gene may be isolated from cells expressing the protein. The identified and isolated nucleic acids may then be ligated into an appropriate cloning vector. A large number of vector/host systems including, but are not limited to, bacteriophages (*e.g.*, lambda derivatives) or plasmids such as pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene; La Jolla, CA), may be utilized in the practice of the present invention. Insertion of the sequence of interest into a cloning vector may be accomplished by: (*i*) ligating the DNA fragment into a cloning vector which has complementary cohesive termini; (*ii*) enzymatic modification of the termini if the complementary restriction sites used to fragment the DNA are not present in the cloning vector;

(*iii*) ligating nucleotide sequences (linkers) onto the DNA termini which comprise specific chemically-synthesized oligonucleotides possessing restriction endonuclease recognition sequences or (*iv*) modification of the insert termini by homopolymeric tailing with terminal deoxynucleotidyl transferase (TdT). The recombinant molecules may be introduced into the host cells via transformation, transfection, infection, electroporation, and the like.

In an alternative embodiment of the present invention, the desired gene may be identified and isolated following its insertion into a suitable cloning vector by use of a "shot-gun" cloning approach. It should be noted, however, that enrichment for the gene of interest may be accomplished, for example, by size fractionation prior to insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated 5 53BP2:IP-l, 53BP2:IP-2 or 535P2-IP-3 gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 sequences disclosed within the present invention include those nucleotide sequences: (*i*) encoding substantially the same amino acid sequences as found in native 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 proteins; (*ii*) encoding amino acid sequences with functionally-equivalent amino acids and (*iii*) encoding other 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 derivatives or analogs.

(3) ANTIBODIES TO 53BP2•53BP2-IP COMPLEXES AND 53BP2:IP-1, 53BP2:IP-2 AND 53BP2:IP-3 PROTEINS

As disclosed by the present invention, the 53BP2•53BP2-IP complexes (*e.g.*, 53BP2-IP complexes with β-tubulin, p62, hnRNP G, 53BP2: IP-l, 53BP2:IP-2 or 53BP2:IP-3 (or fragments, derivatives or homologs thereof), as well as 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein (or fragments, homologs and derivatives thereof) may be utilized as immunogens in the generation of antibodies which immunospecifically-bind such these aforementioned immunogens. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} fragments, and an F_{ab} expression library. In a specific embodiment of the present invention, methodologies for the production of antibodies specific for complexes of the human 53BP2 protein and human 53BP2-IPs are disclosed. In another specific embodiment, complexes formed from fragments of the 53BP2 protein and a 53BP2-IP (wherein the fragments possess the protein domain which interacts with the other member of the complex) are used as immunogens

for the production of antibodies. In yet another specific embodiment of the present invention, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 (or fragments, derivatives, analogs or homologs thereof) are utilized as immunogens.

Various procedures well-known within the art may be used for the production of polyclonal antibodies to a 53BP2•53BP2-IP complex (or derivatives, fragments or analogs thereof) or to a 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein (or derivatives, fragments or analogs thereof). For production of the specific antibody, various host animals may be immunized by injection with the native 53BP2•53BP2-IP complex, or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein, or a synthetic version or a derivative of the foregoing (e.g., a cross-linked 53BP2•53BP2-IP complex). Host animals which may be used in the production of antibodies include, but are not limited to rabbits, mice, rats, and the like. In addition, adjuvants may be utilized to increase the immunological response.

For preparation of monoclonal antibodies directed towards a 53BP2•53BP2-IP complex, or a 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein (or derivatives, fragments or analogs thereof) any technique which provides for the production of antibody molecules by continuous cell lines in *in vitro* culture may be used. Culture techniques include, but are not limited to: (*i*) the hybridoma technique (see *e.g.*, Kohler & Milstein, 1975. *Nature* 256:495-497); (*ii*) the trioma technique; (*iii*) the human B-cell hybridoma technique (see *e.g.*, Kozbor, *et al.*, 1983. *Immunology Today* 4:72) and the EBV hybridoma technique to produce human monoclonal antibodies (see *e.g.*, Cole, *et al.*, 1985. In: *Monoclonal Antibodies and Cancer Therapy* (Alan R. Liss, Inc.). In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing a recently developed technology (see *e.g.*, PCT Patent Publication U590/02545).

As disclosed in the present invention, techniques described for the production of single-chain antibodies (see *e.g.*, U.S. Patent No. 4,946,778) may be adapted to produce 53BP2•53BP2-IP complex-specific and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3-specific single-chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of F_{ab} expression libraries (see *e.g.*, Huse, *et al.*, 1989. *Science* 246:1275- 1281) to allow rapid and efficacious identification of monoclonal F_{ab} fragments with the desired specificity for 53BP2:β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 complexes, or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 (or derivatives or analogs thereof). Non-human antibodies may be "humanized" by known methods (see *e.g.*, U.S. Patent No. 5,225,539).

In the production of antibodies, screening for the desired antibody may be accomplished by techniques known within the art (*e.g.*, enzyme-linked immunosorbent assay; ELISA)). To select antibodies specific for a particular domain of the 53BP2•53BP2-IP complex or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3, one may screen the generated hybridomas for a product which binds to the fragment of the 53BP2•53BP2 complex or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 that possesses such a domain. Similarly, for selection of an antibody which specifically-binds a 53BP2•53BP2-IP complex but does not specifically-bind to the individual proteins of the 53BP2-IP complex, one can select on the basis of positive binding to the 53BP2•53BP2-IP complex and a lack of binding to the individual 53BP2 and 53BP2-IP proteins.

In a preferred embodiment of the present invention, antibodies specific to a domain of the 53BP2•53BP2-IP complex are provided, as are antibodies to specific domains of 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3. The foregoing antibodies may be utilized in methods well-known within the art relating to the localization and/or quantitation of 53BP2•53BP2-IP complexes and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins of the invention (*e.g.*, for imaging these proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, and the like). In another embodiment of the invention anti-53BP2•53BP2-IP complex antibodies and fragments thereof, or anti-53BP2:IP-1, anti-53BP2:IP-2, and anti-53BP2:IP-3 (or derivatives and fragments thereof).

(4) DIAGNOSTIC, PROGNOSTIC, AND SCREENING USES OF 53BP2•2-IP COMPLEXES AND 53BP2:IP-1, 53BP2:IP-2 AND 53BP2:IP-3 PROTEINS

53BP2•53BP2-IP complexes (particularly the 53BP2 protein complexed with β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3), as well as 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 proteins, may function as "markers" of specific disease states involving the disruption of cell cycle progression, cellular apoptosis and/or differentiation, intracellular signal transduction, protein transport, and/or c-*Src* activation, transcriptional or translational regulation, tumorigenesis and tumor progression, ubiquitin-mediated proteolysis, mRNA binding and metabolism, and effects on autoimmune processes, and thus have potential diagnostic utility. Further, classification and differentiation of particular groups of patients with elevations or deficiencies of a 53BP2•53BP2-IP complex, as well as the 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein may potentially lead to new nosological classifications of diseases, furthering diagnostic ability.

Detecting levels of 53BP2•53BP2-IP complexes, or individual proteins that have been shown to form complexes with 53BP2, or the 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 proteins, or detecting levels of the mRNA encoding the components of the 53BP2•53BP2-IP complexes, or the 53BP2: IP-l, 53BP2:IP-2 or 53BP2:IP-3 proteins, may be utilized in prognosis, to follow the course of disease states, to follow therapeutic response, and the like. 53BP2•53BP2-IP complexes and the individual components of the 53BP2•53BP2-IP complexes (*e.g.*, 53BP2, B-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, and derivatives, analogs and sub-sequences thereof) 53BP2 and/or 53BP2-IP, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 nucleic acids (and sequences complementary thereto), and anti-53BP2•53BP2-IP complex antibodies and antibodies directed against the individual components which can form 53BP2•53BP2-IP complexes and anti-53BP2:IP-1, anti-53BP2:IP-2 and anti-53BP2:IP-3 antibodies, have potential uses in diagnostics. These aforementioned molecules may be utilized used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders characterized by aberrant levels of 53BP2•53BP2-IP complexes or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 proteins, or monitor the treatment thereof. In a specific embodiment of the present invention, a 53BP2•53BP2-IP complex is detected that *is not* a complex of the 53BP2 protein and PP1α or p53.

In one embodiment of the present invention, the immunoassay methodology is comprised of contacting a sample derived from a patient with an anti-53BP2•53BP2-IP complex antibody or an anti-53BP2:IP-l, anti-53BP2:IP-2 or anti-53BP2:IP-3 antibody under conditions such that

immunospecific binding may occur, and subsequently detecting or measuring the amount of any immunospecific binding by the aforementioned antibodies. In a specific embodiment, the binding of antibody (within tissue sections) may be utilized to detect aberrant localization of the 53BP2•53BP2-IP complex or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 protein and/or aberrant (*i.e.*, high, low or absent) levels of the 53BP2•53BP2-IP complex or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 protein. In another specific embodiment of the present invention, an antibody specific for the 53BP2•53BP2-IP complex may be used to screen a patient tissue or serum sample for the presence of 53BP2•53BP2-IP complex; wherein an aberrant level of the 53BP2•53BP2-IP complex is indicative of a pathological condition. In yet another embodiment, antibodies specific for the 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 proteins may be utilized to screen a patient tissue or serum sample for the presence of these aforementioned proteins; wherein an aberrant level of 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 proteins is an indication of a diseased condition. "Aberrant levels," as used herein, is defined as increased or decreased levels of the protein(s) relative to a standard level representing those levels which are present in an analogous sample from a portion of the body or from a subject not having the disease or disorder. The immunoassays which may be used in the practice of the present invention include, but are not limited to, competitive and non-competitive immunoassay systems using methodologies techniques such as Western blots, radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, and the like.

Nucleic acids encoding the components of the 53BP2•53BP2-IP complexes (*e.g.*, the 53BP2 protein, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3), as well as the 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 proteins and related nucleic acid sequences and subsequences (including complementary sequences), may also be used in hybridization assays. The 53BP2 protein, 53BP2-IP, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 nucleic acid sequences (or subsequences thereof comprising at least 8 nucleotides) may be used as hybridization probes. Hybridization assays may be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant levels of the mRNAs encoding the components of a 53BP2•53BP2-IP complex or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 protein as described, *supra*. In a preferred embodiment, the hybridization assay is carried out using nucleic acid probes capable of hybridizing to the 53BP2 protein and to a binding partner of the 53BP2

protein (*e.g.*, β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3) to measure concurrently the expression of both members of a 53BP2•53BP2-IP complex.

(5) THERAPEUTIC UTILIZATION OF 53BP2•53BP2-IP COMPLEXES AND 53BP2:IP-1, 53BP2:IP-2 AND 53BP2:IP-3

The present invention provides for treatment or prevention of various diseases and disorders by administration of a therapeutic compound (hereinafter defined as a "Therapeutic"). Such Therapeutics include, but are not limited to: (*i*) 53BP2•53BP2-IP complexes (*e.g.*, the 53BP2 protein complexed with β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3), the 53BP2 protein alone and the individual 53BP2-IPs proteins (*e.g.*, β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3) and derivatives, fragments and analogs of the foregoing; (*ii*) antibodies thereto; (*iii*) nucleic acids encoding the 53BP2 protein and/or the 53BP2-IP and 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, and analogs or derivatives, thereof; (*iv*) 53BP2, 53BP2-IP, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 antisense nucleic acids and (*v*) 53BP2•53BP2-IP complex and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 modulators (*i.e.*, inhibitors, agonists and antagonists).

The 53BP2 protein and several of its binding partners, as identified herein, (*e.g.*, β-tubulin, p62 and hnRNP G) are implicated in disorders of cell cycle progression, cell differentiation, and transcriptional control, including cancer and tumorigenesis and tumor progression. Disorders of neurodegeneration resulting from altered cellular apoptosis, mRNA destabilization and ubiquitin-mediated proteolysis, may also involve these same proteins. For example, HnRNP G is specifically implicated in autoimmune disorders. A wide range of cell diseases affected by intracellular signal transduction, including c-*Src* signaling, and translational regulation may be therapeutically or prophylactically treated by the administration of a Therapeutic which modulates (*i.e.*, inhibits, antagonizes or promotes) the activity of the 53BP2•53BP2-IP complex, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3. Similarly, diseases and disorders associated with aberrant levels of 53BP2•53BP2-IP complex levels or activity or aberrant levels of the 53BP2, 53BP2-IP, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 proteins may be treated or prevented by the administration of a Therapeutic which modulates 53BP2•53BP2-IP complex formation or activity or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 activity. In a specific embodiment of the present , the activity or levels of the 53BP2 protein is modulated by administration of a 53BP2-IP. Diseases and disorders which are characterized by either increased or decreased (relative to a subject not suffering from the disease or disorder)

53BP2•53BP2-IP levels or activity, or increased or decreased 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 levels or activity, may be treated by the administration of a Therapeutics which antagonize (*i.e.*, reduces or inhibits) or increases 53BP2•53BP2-IP complex formation or activity, or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 levels or activity, respectively.

Therapeutics which can be used include, but are not limited to: (*i*) the 53BP2 or a 53BP2-IP proteins (or analogs, derivatives or fragments thereof); (*ii*) anti-53BP2•53BP2-IP complex antibodies (*e.g.*, antibodies which are specific for complexes of the 53BP2 protein with β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 complexes) and fragments and derivatives thereof containing the binding region thereof; (*iii*) nucleic acids encoding the 53BP2 or 53BP2-IP proteins; (iv) concurrent administration of 53BP2 and a 53BP2-IP antisense nucleic acid or a 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 anti-sense nucleic acid and (*v*) 53BP2 and/or 53BP2-IP, or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 nucleic acids, which are dysfunctional (*e.g.*, due to a heterologous non-53BP2, non-53BP2-IP, non-53BP2:IP-l, non-53BP2:IP-2 and/or non-53BP2:IP-3 insertion within the aforementioned proteins coding sequences) which are used to "knockout" endogenous 53BP2, 53BP2-IP, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 function by homologous recombination (see *e.g.*, Capecchi, 1989. *Science* 244:1288-1292).

In specific embodiments of the present invention, nucleic acids containing a portion of a 53BP2, 53BP2-IP, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 gene in which these aforementioned sequences flank (*i.e.*, are both 5' and 3' to) a different gene sequence, are utilized as antagonists so as to promote 53BP2, 53BP2-IP, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 inactivation by homologous recombination. See *e.g.*, Koller & Smithies, 1989. *Proc. Natl. Acad. Sci. USA* 86:8932-8935. In an additional embodiment, mutants or derivatives of a first 53BP2-IP protein which possesses greater affinity for the 53BP2 protein than the wild-type, first 53BP2-IP may be administered to compete with a second 53BP2-IP protein for binding to the 53BP2 protein, thereby reducing the levels of complexes which contain the 53BP2 protein with the second 53BP2-IP.

Other Therapeutics which inhibit 53BP2•53BP2-IP complex or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 function may be identified by use of *in vitro* assays which are well-known within the art. In specific embodiments of the present invention, Therapeutics which antagonize 53BP2•53BP2-IP complex formation and/or activity or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 activity are administered in a therapeutic or prophylactic manner: (*i*) in diseases or disorders involving an increased (*i.e.*, relative to normal or desired) level of 53BP2•53BP2-IP complex or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3, for example, in patients where these complexes or

proteins are overactive or over-expressed or (*ii*) in diseases or disorders wherein *in vitro* or *in vivo* assays indicate the utility of 53BP2•53BP2-IP complex or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 antagonist administration. Increased levels of 53BP2-associated complexes or proteins may be readily detected (*e.g.*, by quantifying protein, RNA or cDNA generated from RNA) by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and performing *in vitro* screening for RNA or protein levels, structure and/or activity of the expressed 53BP2•53BP2-IP complex (or the 53BP2 and 53BP2-IP mRNA) or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein or mRNA. Various methodlogies within the art may be employed including, but not limited to: (*i*) immunoassays to detect and/or visualize 53BP2•53BP2-IP complexes or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 (*e.g.*, via Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry and the like) and/or hybridization assays to detect concurrent expression of 53BP2, 53BP2-IP, 53BP2:IP-1, .53BP2:IP-2 or 53BP2:IP-3 mRNA (*e.g.*, via Northern assays, dot blots, *in situ* hybridization and the like). A specific embodiment of the present invention includes methods of reducing 53BP2•53BP2-IP complex expression (*i.e.*, the expression of the two components of the 53BP2•53BP2-IP complex and/or formation of the complex) or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 expression, by targeting mRNAs which express these protein moieties. RNA therapeutics is currently differentiated into three classes: antisense species, ribozymes or RNA aptamers. See *e.g.*, Good, *et al.*, 1997. *Gene Therapy* 4:45-54. Antisense oligonucleotides have been the mode most widely utilized and, by way of example but not of limitation, antisense oligonucleotide methodology to reduce 53BP2 complex formation will be fully disclosed, *infra*. Ribozyme therapy involves the administration, induced expression and the like, of small RNA molecules which possess enzymatic ability to cleave, bind or otherwise inactivate specific RNAs to reduce or eliminate expression of particular proteins. See *e.g.*, Grassi & Marini, 1996. *Ann. Med.* 28:499-510. At present, however, the design of "hairpin" and "hammerhead" RNA ribozymes remains necessary so as to specifically-target a particular mRNA (*e.g.*, the 53BP2 mRNA). RNA aptamers are specific RNA ligands for proteins, such as for Tat and Rev RNA (see *e.g.*, Good, *et al.*, 1997. *Gene Therapy* 4:45-54) that can specifically inhibit their translation. Aptamers specific for the 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 proteins may be identified by many methods which well-known within the art including, but not limited to, the protein-protein interaction assay described, *infra*. In another embodiment, the activity or levels of 53BP2 is reduced by the administration of a 53BP2-IP, a nucleic acid which encodes the 53BP2-IP, an antibody which immunospecifically-binds the 53BP2-IP or a fragment or

derivative of the antibody which contains the binding domain thereof. Additionally, the levels or activity of a 53BP2-IP maybe reduced by administration of 53BP2, a nucleic acid which encodes 53BP2, an antibody which immunospecifically-binds 53BP2 or a fragment or derivative of the antibody which contains the binding domain thereof.

In specific embodiment of the present invention, diseases or disorders which are associated with increased levels of 53BP2 or a particular 53BP2-IP (*e.g.*, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3) may be treated or prevented by administration of a Therapeutic which increases 53BP2•53BP2-IP complex formation if the complex formation acts to reduce or inactivate 53BP2 or the particular 53BP2-IP through the 53BP2•53BP2-IP complex formation. Such diseases or disorders can be treated or prevented by administration of one member of the 53BP2•53BP2-IP complex, including mutants of a member of the 53BP2•53BP2-IP complex that has increased affinity for the other member of the 53BP2•53BP2-IP complex (so as to cause increased complex formation), administration of antibodies or other molecules that stabilize the 53BP2•53BP2-IP complex and the like. Diseases and disorders associated with under-expression of a 53BP2•53BP2-IP complex, 53BP2, 53BP2-IP, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 may be treated or prevented by the administration of a Therapeutic which promotes (*i.e.*, increases or supplies) 53BP2•53BP2-IP complex or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 function. Examples of such a Therapeutic include, but are not limited to, 53BP2•53BP2-IP complexes and 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 proteins and derivatives, analogs and fragments thereof which are functionally active (*i.e.*, possess the ability to form 53BP2•53BP2-IP complexes), non-complexed 53BP2 and 53BP2-IP proteins and derivatives (and analogs or fragments thereof) and nucleic acids encoding the members of a 53BP2•53BP2-IP complex or encoding 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3, or functionally active derivative or fragment thereof (*e.g.*, for use in gene therapy).

In other specific embodiments of the present invention, Therapeutics which promote 53BP2•53BP2-IP complex or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 function, are administered in a therapeutic or prophylactic manner: (*i*) in diseases or disorders involving an absence or decreased (*i.e.*, relative to normal or desired) level of 53BP2•53BP2-IP complex or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 proteins, for example, in patients where the complexes (or the individual components necessary to form the complexes) or proteins are lacking, genetically defective, biologically inactive or under-active, or under-expressed or (*ii*) in diseases or disorders wherein *in vitro* or *in vivo* assays indicate the utility of 53BP2•53BP2-IP complex or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 agonist administration. The absence or decreased level in

53BP2•53BP2-IP complex or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein and/or function may be readily detected (*e.g.*, by obtaining a patient tissue sample from biopsy tissue) and performing *in vitro* screening for RNA, cDNA generated from mRNA, protein levels, structure and/or activity of the expressed 53BP2•53BP2-IP complex (or for the concurrent expression of mRNA encoding the two components of the 53BP2•53BP2-IP complex) or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 RNA or protein. Various methodologies well-known within the art may be employed to detect and/or visualize 53BP2•53BP2-IP complexes (or the individual components of 53BP2•53BP2-IP complexes) or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 (*e.g.*, via Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry and the like) and/or hybridization assays to detect expression of the mRNA encoding the individual protein components of the 53BP2•53BP2-IP complexes or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 by detecting and/or visualizing 53BP2 and a 53BP2-IP concurrently, or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 mRNA (*e.g.*, Northern assays, dot blots, in situ hybridization, and the like).

In specific embodiment of the present invention, the activity or levels of 53BP2 are increased by administration of a 53BP2-IP (or a derivative or analog thereof), a nucleic acid encoding a 53BP2-IP, or an antibody which immunospecifically-binds a 53BP2-IP, or a fragment or derivative of the antibody which possesses the binding domain thereof. In another specific embodiment, the activity or levels of a 53BP2-IP are increased by administration of 53BP2 (or a derivative or analog thereof), a nucleic acid encoding 53BP2, or an antibody which immunospecifically-binds 53BP2. or a fragment or derivative of the antibody which possesses the binding domain thereof. Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, a human 53BP2•53BP2-IP complex or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein (or a derivative or analog thereof), nucleic acids encoding the members of the human 53BP2•53BP2-IP complex or human 53BP2:IP-1, human 53BP2:IP-2 or human 53BP2:IP-3 (or a derivative or analog thereof), an antibody to a human 53BP2•53BP2-IP complex or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 (or a derivative thereof), is therapeutically or prophylactically administered to a human patient. Preferably, suitable *in vitro* or *in vivo* assays are utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue. In various specific embodiments, *in vitro* assays may be performed with representative cells of the specific cell type(s) involved in a patient's disorder so as to determine whether a given Therapeutic has the desired effect upon such cell

types. Prior to administration to humans, *in vivo* testing may be performed utilizing any animal model system known within the art.

(6) MALIGNANCIES

Various components of the 53BP2•53BP2-IP complexes (*i.e.*, 53BP2, β-tubulin, p62 and hnRNP G) have been implicated in the regulation of cell proliferation. Accordingly, Therapeutics of the present invention may prove useful in treating or preventing diseases or disorders associated with cell over-proliferation or loss of control of cell proliferation, particularly cancers, malignancies and tumors. Therapeutics of the present invention may be assayed by any method known within the art for efficacy in treating or preventing malignancies and related disorders. Such assays include, but are not limited to, *in vitro* assays using transformed cells or cells derived from a tumor of a patient or *in vivo* assays using animal models of cancer or malignancies, or any of the assays described, *infra*. Potentially effective Therapeutics may function, but are not limited, to inhibit proliferation of tumor or transformed cells in culture or cause regression of tumors in animal models in comparison to controls. Accordingly, once a malignancy has been found to be amenable to treatment by modulation (*i.e.*, inhibit, antagonize or agonize) of 53BP2•53BP2-IP complex activity, or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 activity, that specific malignancy may be treated or prevented by administration of a Therapeutic which modulates the formation of the 53BP2•53BP2-IP complex (*e.g.*, supplying 53BP2•53BP2-IP complexes and the individual binding partners of a 53BP2•53BP2-IP complex, such as 53BP2, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3), or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 function.

For example, in specific embodiments of the present invention, malignancy or dysproliferative changes (*i.e.*, metaplasias and dysplasias) or hyperproliferative disorders, are treated or prevented in the bladder, breast, colon, lung, melanoma, pancreas, or uterus. In other specific embodiments, sarcoma, or leukemia is treated or prevented.

(7) PRE-MALIGNANT CONDITIONS

The Therapeutics of the invention that are effective in treating cancer or malignancies (*e.g.* as described above) may also be administered so as to treat pre-malignant conditions and to prevent their possible progression to a neoplastic or malignant state. Such prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular where non-neoplastic cell growth (*i.e.*, hyperplasia, metaplasia

or, most particularly, dysplasia) has occurred. See *e.g.*, Robbins & Angell, 1976. *Basic Pathology, 2d Ed.* (W.B. Saunders Co., Philadelphia, PA).

Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. One example, endometrial hyperplasia, often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of fully-differentiated (*i.e.*, adult) cell substitutes for another type of fully-differentiated cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia, frequently a forerunner of cancer, is found mainly in the epithelia and is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder. Alternatively, or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, may be indicative of the desirability of prophylactic/ therapeutic administration of a Therapeutic of the present invention which modulates 53BP2•53BP2-IP complex activity or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 activity.

The characteristics of a transformed phenotype include, but are not limited to, morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250 kD cell surface protein and the like. In a specific embodiment of the present invention, leukoplakia (a benign-appearing hyperplastic or dysplastic lesion of the epithelium) or Bowen's disease (a carcinoma *in situ*) are pre-neoplastic lesions which are amenable to prophylactic intervention by administration of a Therapeutic of the present invention. In another specific embodiment, fibrocystic disease (*e.g.*, cystic hyperplasia, mammary dysplasia, adenosis, and benign epithelial hyperplasia) is also amenable to prophylactic intervention.

In other embodiments of the present invention, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an therapeutically-effective amount of a Therapeutic: (*i*) a chromosomal translocation associated with a malignancy (*e.g.*, the bcr/*abl* translocation in chronic myelogenous leukemia; (*ii*) t(14,18)

for follicular lymphoma; (*iii*) familial polyposis or Gardner's syndrome (possible precursors of colon cancer); (*iv*) monoclonal gammopathy of undetermined significance (MGUS; a possible precursor of multiple myeloma) and (*v*) a first degree kinship with persons having a cancer or pre-cancerous disease showing a Mendelian inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome). See *e.g.*, Robbins & Angell, 1976. *Basic Pathology, 2d Ed.* (W.B. Saunders Co., Philadelphia, PA).

In yet another specific embodiment, a Therapeutic of the present invention is administered to a human patient to prevent progression to breast, colon, lung, pancreatic, or uterine cancer, or melanoma or sarcoma.

(8) HYPERPROLIFERATIVE AND DYSPROLIFERATIVE DISORDERS

In another specific embodiment of the present invention, a Therapeutic is administered so to treat or prevent hyperproliferative or benign dysproliferative disorders. Therapeutics of the present invention may be assayed by any method known within the art for efficacy in treating or preventing hyperproliferative diseases or disorders (*e.g., in vitro* cell proliferation assays, *in vitro* or *in vivo* assays utilizing animal models of hyperproliferative diseases or disorders or the like). Functions of potentially effective Therapeutics include, but are not limited to, promoting cell proliferation in *in vitro* culture or causing growth or cell proliferation in animal models in comparison to controls. Accordingly, once a hyperproliferative disorder has been shown to be amenable to treatment by modulation of 53BP2•53BP2-IP complex activity or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 activity, that hyperproliferative disease or disorder may be treated or prevented by administration of a Therapeutic which modulates 53BP2•53BP2-IP complex formation (*e.g.*, supplying 53BP2•53BP2-IP complexes and the individual binding partners of a 53BP2•53BP2-IP complex, such as 53BP2, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3) or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 function. Specific embodiments of the present invention are directed towards the treatment or prevention of hepatic cirrhosis (a condition in which scarring has overtaken normal liver regeneration processes); treatment of keloid or hypertrophic scar formation (causing a disfiguring of the skin in which the scarring process interferes with normal renewal); psoriasis (a common skin condition characterized by

excessive proliferation of the skin and delay in proper cell-fate determination); benign tumors; fibrocystic conditions and tissue hypertrophy (*e.g.*, prostatic hyperplasia).

(9) NEURODEGENERATIVE DISORDERS

The 53BP2 protein and certain binding partners thereof (*e.g.*, β-tubulin and p62) have been implicated in the deregulation of cellular maturation and apoptosis, which are characteristic of neurodegenerative disease. Accordingly, Therapeutics of the present invention, particularly those which modulate the levels or activity of 53BP2•β-tubulin or 53BP2•p62 complexes, may be effective in treating or preventing neurodegenerative diseases or disorders. These Therapeutics may be screened for efficacy in treating or preventing such neurodegenerative diseases and disorders by any assay known within the art including, but not limited to, *in vitro* assays for regulated cell maturation or inhibition of apoptosis or *in vivo* assays using animal models of neurodegenerative diseases or disorders. Potentially effective Therapeutics, for example but not by way of limitation, promote regulated cell maturation and prevent cell apoptosis in culture or reduce neurodegeneration in animal models in comparison to controls. Once a neurodegenerative disease or disorder has been shown to be amenable to treatment by modulation of 53BP2•53BP2-IP complex activity or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 activity, that specific neurodegenerative disease or disorder may be treated or prevented by administration of a Therapeutic which modulates 53BP2•53BP2-IP complex formation (including supplying 53BP2•53BP2-IP complexes, such as 53BP2•β-tubulin and 53BP2•p62 complexes) or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 function.

(10) AUTOIMMUNE DISORDERS

The 53BP2-interacting protein, hnRNP G, has been implicated in autoimmune disorders. Therapeutics of the present invention, particularly those which modulate or supply 53BP2•hnRNP G complex activity may be effective in treating or preventing autoimmune diseases or disorders. Therapeutics which are effective in treating or preventing such autoimmune diseases and disorders may be screened for such efficacy by any assay known within the art including, but not limited to, *in vitro* assays for using cell culture models or *in vivo* assays using animal models of autoimmune diseases or disorders. Potentially effective Therapeutics, for example but not by way of limitation, reduce autoimmune responses in animal models in comparison to controls. Accordingly, once an autoimmune disease or disorder has been shown to be amenable to treatment by modulation of 53BP2•53BP2-IP complex activity or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 activity, that autoimmune disease or disorder may be

treated or prevented by administration of a Therapeutic which modulates 53BP2•53BP2-IP complex formation (including supplying 53BP2•53BP2-IP complexes) or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 function.

(11) GENE THERAPY

In embodiments of the present invention, nucleic acids comprising a sequence encoding 53BP2 and a 53BP2-IP, or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 (or functional derivatives thereof) are administered to modulate 53BP2•53BP2-IP complex or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 function, by way of gene therapy. In specific embodiments, a nucleic acid or nucleic acids encoding both 53BP2 and a 53BP2-IP (*e.g.*, β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3) or functional derivatives thereof, are administered by way of gene therapy. Gene therapy, as utilized herein, refers to therapy performed by the administration of a nucleic acid to a subject. In this embodiment of the present invention, the nucleic acid produces its encoded protein(s) which serves to mediate a therapeutic effect by modulating 53BP2•53BP2-IP complex or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 function.

Any of the methods for gene therapy known within the art may be utilized in the practice of the present invention. See *e.g.*, Goldspiel, *et al.*, 1993. *Clin. Pharmacy* 12:488-505; Wu & Wu, 1991. *Biotherapy* 3: 87-95; Mulligan, 1993. *Science* 260:926-932. In a preferred embodiment of the present invention, the Therapeutic comprises a 53BP2 and a 53BP2-IP nucleic acid or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 nucleic acid which is part of an expression vector that expresses the proteins 53BP2 and a 53BP2-IP or expresses 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 (or fragments or chimeric proteins thereof) in a suitable host. In particular, such a nucleic acid possesses a promoter operably-linked to the 53BP2 and the 53BP2-IP coding region(s) or, less preferably, two separate promoters operably-linked to the 53BP2 and the 53BP2-IP coding regions separately or linked to the 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 coding region, wherein said promoter is inducible or constitutive and, optionally, tissue-specific.

In another particular embodiment, a nucleic acid molecule is used in which the 53BP2 and 53BP2-IP coding sequences or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 coding sequences, and any other desired sequences, are flanked by regions which promote homologous recombination at a desired site in the genome, thus providing for intra-chromosomal expression of the 53BP2 and the 53BP2-IP nucleic acids or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 nucleic acids. See *e.g.*, Koller & Smithies, 1989. *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra, *et*

al., 1989. *Nature* 342:435-438. Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy. In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This may be accomplished by any of numerous methods known in the art including, but not limited to, constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular by: (*i*) infection using a defective or attenuated retroviral or other viral vector (see *e.g.*, U.S. Patent No. 4,980,286); (*ii*) direct injection of naked DNA; (*iii*) use of microparticle bombardment (*e.g.*, a gene gun - Biolistic, DuPont); (*iv*) coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules; (*v*) by administering it in linkage to a peptide which is known to enter the nucleus; (*vi*) administering it in linkage to a ligand subject to receptor-mediated endocytosis (see *e.g.*, Wu & Wu, 1987. *J. Biol. Chem.* 262:4429-4432) which can be used to target cell types specifically-expressing the receptors and the like.

In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid may be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor. See *e.g.*, PCT Publications W0 93/14188; WO 93/20221. Alternatively, the nucleic acid may be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination. See *e.g.*, Koller & Smithies, 1989. *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra, *et al.*, 1989. *Nature* 342:435-438. In a specific embodiment, a viral vector which contains the 53BP2 and/or the 53BP2-IP nucleic acids or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 nucleic acid is utilized. For example, a retroviral vector may be used (see *e.g.*, Miller, *et al.*, 1993. *Meth. Enzymol.* 217:581-599) which have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The 53BP2 and/or 53BP2-IP (preferably both 53BP2 and 53BP2-IP) nucleic acids or 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 nucleic acids, to be used in gene therapy is/are cloned into the vector, which facilitates delivery of the gene into a patient.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia where the virus

naturally infect to cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. In addition, adenoviruses have the advantage of being capable of infecting non-dividing cells. See *e.g.*, Kozarsky & Wilson, 1993. *Curr. Opin. Genet. Develop.* 3:499-503. Adeno-associated virus (AAV) has also been proposed for use in gene therapy. See *e.g.*, Walsh, *et al.*, 1993. *Proc. Soc. Exp. Biol. Med.* 204:289-300. Another approach to gene therapy involves transferring a gene into cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate-mediated transfection, or viral infection. Generally, the method of transfer includes the transfer of a selectable marker to the cells which are then placed under selection to isolate those cells which have taken-up and are expressing the transferred gene and only those selected cells are then delivered to a patient. In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art including, but not limited to, transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, and the like (see *e.g.*, Loeffler & Behr, 1993. *Meth. Enzymol.* 217:599-618; Cohen, *et al.*, 1993. *Meth. Enzymol.* 217:618-644) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique chosen should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny. The resulting recombinant cells can be delivered to a patient by various methods known in the art.

In a preferred embodiment, epithelial cells are injected (*e.g.*, subcutaneously). In another embodiment, recombinant skin cells may be applied as a skin graft onto the patient. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and may be determined by one skilled within the art. Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type and include, but are not limited to: epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes, blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes, various stem or progenitor cells, in particular hematopoietic stem or progenitor cells (*e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc).

In a preferred embodiment, the cell used for gene therapy is autologous to the patient. In an embodiment in which recombinant cells are used in gene therapy, a 53BP2 and/or a 53BP2-IP (preferably both a 53BP2 and a 53BP2-IP) nucleic acid or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 nucleic acid is/are introduced into the cells such that the gene or genes are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention. Such stem cells include, but are not limited to, hematopoietic stem cells (HSC), stem cells of epithelial tissues such as the skin and the lining of the gut, embryonic heart muscle cells, liver stem cells (see *e.g.*, PCT Publication WO 94/08598) and neural stem cells (see *e.g.*, Stemple & Anderson, 1992. *Cell* 71:973-985).

Epithelial stem cells (ESCs) or keratinocytes can be obtained from tissues such as the skin and the lining of the gut by known procedures. See *e.g.*, Rheinwald, 1980. *Meth. Cell Bio.* 21:229-237. In stratified epithelial tissue such as the skin, renewal occurs by mitosis of stem cells within the germinal layer, the layer closest to the basal lamina. Stem cells within the lining of the gut provide for a rapid renewal rate of this tissue. ESCs or keratinocytes obtained from the skin or lining of the gut of a patient or donor can be grown in tissue culture. See *e.g.*, Pittelkow & Scott, 1986. *Mayo Clinic Proc.* 61:771-782. If the ESCs are provided by a donor, a method for suppression of host versus graft reactivity (*e.g.*, irradiation, drug or antibody administration to promote moderate immunosuppression) may also be used. With respect to hematopoietic stem cells (HSC), any technique which provides for the isolation, propagation, and maintenance *in vitro* of HSCs may be used in this embodiment of the present invention. Techniques by which this may be accomplished include, but are not limited to: (*i*) the isolation and establishment of HSC cultures from bone marrow cells isolated from the future host, or a donor or (*ii*) the use of previously established long-term HSC cultures, which may be allergenic or xenogeneic. Non-autologous HSC are used preferably in conjunction with a method of suppressing transplantation immune reactions of the future host/patient. In a specific embodiment of the present invention, human bone marrow cells can be obtained from the posterior iliac crest by needle aspiration. See *e.g.*, Kodo, *et al.*, 1984. *J. Clin. Invest.* 73:1377-1384.

In a preferred embodiment, the HSCs may be made highly enriched or in substantially pure form. This enrichment may be accomplished before, during or after long-term culturing, and may be performed by any techniques known in the art. Long-term cultures of bone marrow cells may be established and maintained by using, for example, modified Dexter cell culture

techniques (see Dexter, *et al.*, 1977. *J. Cell Physiol.* 91:335) or Witlock-Witte culture techniques (see Witlock & Witte, 1982. *Proc. Natl. Acad. Sci. USA* 79:3608-3612). In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Additional methods can be adapted for use to deliver a nucleic acid encoding the 53BP2 and/or 53BP2-IP proteins or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 proteins, or functional derivatives thereof.

(12) USE OF ANTISENSE OLIGONUCLEOTIDES FOR SUPPRESSION OF 53BP2•53BP2-IP COMPLEXES AND 53BP2:IP-1, 53BP2:IP-2 AND 53BP2:IP-3

In a specific embodiment of the present invention, 53BP2•53BP2-IP complex function or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein function is inhibited by use of antisense nucleic acids for 53BP2 and/or a 53BP2-IP (*e.g.*, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3), preferably both 53BP2 and the 53BP2-IP or antisense nucleic acids for 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3. The present invention provides the therapeutic or prophylactic use of nucleic acids of at least six nucleotides which are antisense to a gene or cDNA encoding 53BP2 and/or a 53BP2-IP or encoding 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3, or portions thereof. A 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 "antisense" nucleic acid as used herein refers to a nucleic acid capable of hybridizing to a portion of a 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 RNA (preferably mRNA) by virtue of some sequence complementarity. The antisense nucleic acid may be complementary to a coding and/or noncoding region of a 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 mRNA. Such antisense nucleic acids have utility as Therapeutics which inhibit 53BP2•53BP2-IP complex formation or activity or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 function or activity, and may be used in the treatment or prevention of disorders as described, *supra*.

The antisense nucleic acids of the present invention may be oligonucleotides that are double-stranded or single-stranded RNA or DNA (or a modification or derivative thereof) which can be directly administered to a cell, or which can be produced intracellularly by transcription of exogenous, introduced sequences. In another embodiment, the invention is directed to methods for inhibiting the expression of 53BP2 and a 53BP2-IP nucleic acid sequence or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising an antisense nucleic

acid of 53BP2 and 53BP2-IP, or an antisense nucleic acid of 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 (or derivatives thereof) of the present invention.

The 53BP2, 53BP2-IP, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides (ranging from 6 to about 200 oligonucleotides). In specific embodiments, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides may be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide may be modified at the base moiety, sugar moiety, or phosphate backbone. In addition, the oligonucleotide may include other appending groups such as: (*i*) peptides or agents facilitating transport across the cell membrane (see *e.g.*, PCT Publication No. WO 88/09810) or blood-brain barrier (see *e.g.*, PCT Publication No. WO 89/10134); (*ii*) hybridization-triggered cleavage agents (see *e.g.*, Krol, *et al.*, 1988. *BioTechniques* 6:958- 976) or (*iii*) intercalating agents (see *e.g.*, Zon, 1988. *Pharm. Res.* 5:539-549). In a preferred aspect of the invention, a 53BP2, 53BP2-IP, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 antisense oligonucleotide is provided, preferably as single-stranded DNA. In yet another embodiment, the oligonucleotide is an anomeric oligonucleotide, forming specific double-stranded hybrids with complementary RNA in which (contrary to the usual B-units) the strands run parallel to one another. See *e.g.*, Gautier, *et al.*, 1987. *Nucl. Acids Res.* 15:6625-6641. The oligonucleotides may also be conjugated to another molecule (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc).

Oligonucleotides of the present invention may be synthesized by standard methods known in the art, for example, by use of an automated DNA synthesizer such as are commercially available from Biosearch, Applied Biosystems. As an example, but not of a limitation, phosphorothioate oligonucleotides may be synthesized by the method of Stein, *et al.*, 1988. *Nucl. Acids Res.* 16:3209); 5-methylphosphonate oligonucleotides may be prepared by use of controlled pore glass polymer supports (Sarin, *et al.*, 1988. *Proc. Natl, Acad. Sci. U.S.A.* 85:7448-7451), and the like. In a specific embodiment, the 53BP2, 53BP2-IP, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3 antisense oligonucleotides comprise catalytic RNAs, or ribozymes. See *e.g.*, Sarver, *et al.*, 1990. *Science* 247:1222-1225. In another embodiment, the oligonucleotide is a 2'-O-methylribonucleotide (see *e.g.*, Inoue, *et al.*, 1987. *Nucl. Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analog (see *e.g.*, Inoue, *et al.*, 1987. *FEBS Lett.* 215:327-330).

In yet another embodiment, the 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 antisense nucleic acids of the present invention are produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding 53BP2, 53BP2-IP (preferably, a 53BP2 and a 53BP2-IP anti-sense nucleic acid), 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 antisense nucleic acids. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. These aforementioned vectors may be constructed by recombinant DNA technology methods standard within the art. The vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequences encoding the 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 antisense RNAs may be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive and include, but are not limited to, the SV40 early promoter region; the promoter contained in the 3' long terminal repeat of Rous sarcoma virus; the Herpesvirus thymidine kinase promoter; the regulatory sequences of the metallothionein gene and the like.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 gene, preferably a human gene. However, absolute complementarity (although preferred) is not required. A sequence said to be "complementary to at least a portion of an RNA," as referred to herein, means a sequence possessing sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex, in the case of double-stranded 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 RNA it may contain and still form a stable duplex (or triplex). One skilled in the art may ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

The 53BP2 and 53BP2-IP antisense nucleic acid or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 antisense nucleic acids can be used to treat (or prevent) disorders of a cell type which expresses, or preferably over-expresses, the 53BP2•53BP2-IP complex or 53BP2:IP-1,

53BP2:IP-2 or 53BP2:IP-3 protein. In a preferred embodiment, a single-stranded DNA antisense 53BP2 and 53BP2-IP antisense oligonucleotide, or single-stranded DNA antisense 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 oligonucleotide, is used. Cell types which express or overexpress 53BP2 and 53BP2-IP RNA, or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 RNA, may be identified by various methods known in the art including, but are not limited to, hybridization with 53BP2- and 53BP2-IP-specific nucleic acids, or 53BP2:IP-1-, 53BP2:IP-2- or 53BP2:IP-3-specific nucleic acids (*e.g.*, by Northern hybridization, dot blot hybridization, *in situ* hybridization, etc.) or by observing the ability of RNA from the cell type to be translated *in vitro* into 53BP2 and the 53BP2-IP, or into 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3, by immunohistochemistry.

In a preferred embodiment of the present invention, primary tissue from a patient may be screened for 53BP2 or 53BP2-IP expression, or for 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 expression, prior to treatment. Pharmaceutical compositions of the present invention comprising an effective amount of a 53BP2 and a 53BP2-IP antisense nucleic acid, or a 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 antisense nucleic acid in a pharmaceutically-acceptable carrier, may be administered to a patient having a disease or disorder which is of a type that expresses or over-expresses 53BP2•53BP2-IP complexes or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 RNA or protein. The amount of antisense nucleic acid which will be effective in the treatment of a particular disorder or condition will depend upon the nature of the disorder or condition, and may be quantitatively determined by standard clinical techniques. Where possible, it is preferable to determine the antisense cytotoxicity *in vitro*, and then in useful animal model systems prior to testing and use in humans. In a specific embodiment of the present invention, pharmaceutical compositions comprising 53BP2 and 53BP2-IP antisense nucleic acids, or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 antisense nucleic acids, are administered via liposomes, microparticles or microcapsules. In various embodiments of the invention, it may be useful to use such compositions to achieve sustained release of the 53BP2 and 53BP2-IP antisense nucleic acids, or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 antisense nucleic acids. In another specific embodiment, it may be desirable to utilize liposomes targeted via antibodies to specific identifiable central nervous system cell types. See *e.g.*, Leonetti, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87:2448-2451.

(13) ASSAYS OF 53BP2•53BP2-IP COMPLEXES, 53BP2:IP-1, 53BP2:IP-2 AND 53BP2:IP-3 AND DERIVATIVES AND ANALOGS

The functional activity of 53BP2•53BP2-IP complexes and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins (and derivatives, fragments and analogs thereof) may be assayed by various methods well-known within the art. Potential modulators (*i.e.*, inhibitors, agonists and antagonists) of 53BP2•53BP2 complex activity or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 activity (*e.g.*, anti-53BP2•53BP2-IP complex; anti-53BP2-IP or anti-53BP2:IP-1, anti-53BP2:IP-2 and anti-53BP2:IP-3 antibodies and 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 antisense nucleic acids, may be assayed for their ability to modulate 53BP2•53BP2-IP complex formation and/or activity or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 activity.

As an example, but not of a limitation, in one embodiment of the present invention, where one is assaying for the ability to bind or compete with wild-type 53BP2•53BP2-IP complexes or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3, for binding to an anti-53BP2•53BP2-IP complex antibody or anti-53BP2:IP-1, anti-53BP2:IP-2 or anti-53BP2:IP-3 antibodies, various immunoassays known within the art may be used including, but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein-A assays, immunoelectrophoresis assays, and the like. In one embodiment of the present invention, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Numerous means are known within the art for detecting binding in an immunoassay and are encompassed within the scope of the present invention.

The expression of the 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 genes (both endogenous genes and those expressed from cloned DNA containing these genes) may be detected using techniques known in the art including, but not limited to, Southern hybridization, Northern hybridization, restriction endonuclease mapping and DNA sequence analysis. In addition, polymerase chain reaction amplification (PCR; see *e.g.*, U.S. Patent Nos. 4,683,202, 4,683,195, and 4,889,818; Loh, et al., 1989, *Science* 243:217-220) followed by Southern hybridization or RNase protection with probes specific for 53BP2, 53BP2-IP, or 53BP2:IP-1,

53BP2:IP-2 or 53BP2:IP-3 genes in various cell types. Methods of amplification, other than PCR, are well-known within the art and can be employed in the practice of the present invention.

In one specific embodiment, Southern hybridization may be used to detect genetic linkage of 53BP2, 53BP2-IP, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 gene mutations to physiological or pathological states. Various cell types, at various stages of development, may be characterized for their expression of 53BP2 and a 53BP2-IP (particularly concomitant expression of 53BP2 and 53BP2-IP within the same cells) or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 expression. The stringency of the hybridization conditions for northern or Southern blot analysis can be manipulated to ensure detection of nucleic acids with the desired degree of relatedness to the specific probes used. Modifications to these methods and other methods commonly-known within the art may also be utilized.

Derivatives (*e.g.*, fragments and analogs) of 53BP2-IPs, including 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 (and fragments and other derivatives and analogs of 53BP2-IPs) may be assayed for binding to 53BP2 by any method known in the art, for example, the modified yeast two hybrid assay system, immunoprecipitation with an antibody which binds to 53BP2 in a complex followed by analysis by size fractionation of the immunoprecipitated proteins (*e.g.*, by denaturing or nondenaturing polyacrylamide gel electrophoresis), Western analysis, non-denaturing gel electrophoresis, and the like. One embodiment of the present invention provides a methodology for screening a derivative or analog of 53BP2 for biological activity comprising contacting the derivative or analog of 53BP2 with a protein which is selected from the group consisting of β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3, and detecting the formation of a complex between the aforementioned derivative or analog of 53BP2 and the selected protein, and where detecting formation of the complex indicates that said derivative or analog of 53BP2 possesses biological (*e.g.*, binding) activity. Another embodiment discloses a method for screening a derivative or analog of a protein selected from the group consisting of β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 for biological activity which is comprised of contacting the derivative or analog of the selected protein with 53BP2, and detecting the formation of a complex between said derivative or analog of said protein and 53BP2, wherein detecting the formation of said complex indicates that said derivative or analog of said protein has biological activity.

The present invention also provides methods of modulating the activity of a protein that can participate in a 53BP2•53BP2-IP complex (*e.g.*, 53BP2, β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3) by administration of a binding partner of that protein or

derivative or analog thereof. 53BP2 (and derivatives and analogs thereof, can be assayed for the ability to modulate the activity or levels of a 53BP2-IP by contacting a cell or administering an animal expressing a 53BP2-IP gene with a 53BP2 protein, or a nucleic acid encoding a 53BP2 protein or an antibody that immunospecifically binds the 53BP2 protein or a fragment or derivative of said antibody containing the binding domain thereof and measuring a change in 53BP2-IP levels or activity, wherein a change in 53BP2-IP levels or activity indicates that 53BP2 possesses the ability to modulate 53BP2-IP levels or activity. Alternatively, a 53BP2-IP can be assayed for the ability to modulate the activity or levels of a 53BP2 protein by contacting a cell or administering an animal expressing a gene encoding said protein with 53BP2, or a nucleic acid encoding 53BP2, or an antibody that immunospecifically-binds 53BP2, or a fragment or derivative of said antibody possessing the binding domain thereof, wherein a change in 53BP2 levels or activity indicates that the 53BP2-IP possesses the ability to modulate 53BP2 levels or activity.

53BP2, as well as several of the identified binding partners of 53BP2 (*e.g.*, β-tubulin, p62 protein and hnRNP G have been demonstrated to have roles in the control of cell proliferation and, therefore, cell-transformation and tumorigenesis. Accordingly, the present invention discloses methods for screening 53BP2•53BP2-IP complexes and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins (and fragments, derivatives and analogs thereof) for activity in altering cell proliferation, cell transformation and/or tumorigenesis *in vitro* and *in vivo*. The 53BP2•53BP2-IP complexes, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins (and derivatives, fragments and analogs thereof) may be assayed for activity to alter (*i.e.*, either increase or decrease) cell proliferation in cultured cells *in vitro* using methods which are well-known within the art for measuring cell proliferation. Specific examples of cell culture models include, but are not limited to: lung cancer primary rat lung tumor cells (see *e.g.*, Swafford, *et al.*, 1997. *Mol. Cell. Biol.* 17:1366-1374) and large-cell undifferentiated cancer cell lines (see *e.g.*, Mabry, *et al.*, 1991. *Cancer Cells* 3:53-58); colorectal cell lines for colon cancer (see *e.g.*, Park & Gazdar, 1996. *J. Cell Biochem.* Suppl. 24:131-141); multiple established cell lines for breast cancer (see *e.g.*, Hambly, *et al.*, 1997. *Breast Cancer Res. Treat.* 43:247-258)); continuous human bladder cancer cell lines for genitourinary cancers (see *e.g.*, Ribeiro, *et al.*, 1997. *Int. J. Radiat. Biol.* 72:11-20) and established cell lines for leukemias and lymphomas (see *e.g.*, Drexler, 1994. *Leuk. Res.* 18:919-927).

For example, but not by way of limitation, the present invention discloses methodologies for the assay of cell proliferation comprising; measuring ^{3}H-thymidine incorporation, by direct

cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (*e.g.*, fos, myc) or cell cycle markers, etc. Accordingly, one embodiment of the present invention provides a method of screening 53BP2•53BP2-IP complexes, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins (and fragments, derivatives and analogs thereof) for activity in altering proliferation of cells *in vitro*, which is comprised of contacting the cells with a 53BP2•53BP2-IP complex or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein (or derivative, analog or fragment thereof) measuring the proliferation of cells which have been so contacted, and comparing the proliferation of the cells so contacted with a complex or protein of the invention with the proliferation of cells not so contacted with the complex or protein of the invention, wherein in a change in the level of proliferation in said contacted cells indicates that the complex or protein of the invention possess activity to alter cell proliferation. The 53BP2•53BP2-IP complexes and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins (and derivatives, fragments and analogs thereof) may also be screened for activity in inducing or inhibiting cell transformation (or progression to malignant phenotype) *in vitro*. The complexes and proteins of the invention can be screened by contacting either cells with a normal phenotype (for assaying for cell transformation) or a transformed cell phenotype (for assaying for inhibition of cell transformation) with the complex or protein of the invention and examining the cells for acquisition or loss of characteristics associated with a transformed phenotype (a set of *in vitro* characteristics associated with a tumorigenic ability *in vivo*), including, but not limited to: colony formation in soft agar, a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250 kD surface protein, and the like. See *e.g.*, Luria, *et al.*, 1978. *General Virology, 3d Ed.*, (John Wiley & Sons, New York, NY).

The 53BP2•53BP2-IP complexes and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins (and derivatives, fragments and analogs thereof) may also be screened for activity to promote or inhibit tumor formation *in vivo* in non-human test animal. For example, the complexes and proteins of the invention may be administered to a non-human test animal (preferably a test animal predisposed to develop a type of tumor) and the non-human test animals subsequently examined for an increased incidence of tumor formation in comparison with controls not administered the complex or protein of the invention. Alternatively, the complexes and proteins of the invention can be administered to non-human test animals having tumors (*i.e.*, animals in which tumors have been induced by introduction of malignant, neoplastic, or transformed cells or

by administration of a carcinogen) and subsequently examining the tumors in the test animals for tumor regression in comparison to controls.

The 53BP2•53BP2-IP complexes and 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 proteins, (and derivatives, analogs and fragments thereof) may also be screened for activity in modulating the activity of 53BP2 and the 53BP2 binding partners (*i.e.*, the 53BP2-IPs, particularly β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3) involved in specific 53BP2•53BP2-IP complexes. For example, 53BP2 has been shown to bind a specific domain of the p53 protein and, by virtue of 53BP2-binding, enhance the tumor suppressor activity of p53. Accordingly, the complexes and proteins of the invention can be screened for the ability to modulate (*i.e.*, either increase or decrease) 53BP2 binding to p53 or the 53BP2-binding domain of p53 (see *e.g.*, Naumovski & Cleary, 1996. *Mol. Cell. Biol.* 16:3884-3892) or for the ability to modulate the tumor suppressive activity of p53 by a protein binding assay known within the art (see *e.g.*, Iwabuchi, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91:6098-6102).

53BP2 has also been demonstrated to affect the phosphorylation and dephosphorylation of p53 by 53BP2's binding to protein phosphatase 1 (PPl). Thus, the complexes and proteins of the present invention may be screened by assaying for changes in the level of p53 phosphorylation (see e.g., Milne, *et al.*, 1994. *J. Biol. Chem.* 269:9253-9260) or the level of 53BP2 binding to PPl (*e.g.*, by methods described *supra*). β-tubulin has also been shown to be up-regulated in adenocarcinoma cells and, possibly, to bind proteins with *Src* homology 2 (SH2 domains), such as the PDGF receptor. See *e.g.*, Shaffhausen, 1995. *Biochem. Biophys. Acta* 1242:61-75. Thus, the complexes and proteins of the invention can be screened by assaying for changes in β-tubulin levels (*e.g.*, by immunoassays with anti-β-tubulin antibodies) or for changes in β-tubulin binding to proteins with SH2 domains. Additionally, the protein p62 associates with the p2lwaf GTPase-activating protein (GAP), *Src* family tyrosine kinase 5H3 domains in signaling proteins, binds RNA, interacts with ubiquitin, and also interacts with the cytosolic protein tyrosine kinase that negatively regulates the *Src* family protein kinases. Further, p62 may also play a role in docking certain proteins to the cytoskeleton or membrane upon c-*Src* activation. Thus, the complexes and proteins of the invention may also be screened by measuring changes in p62 binding to GAP (see *e.g.*, Wong, *et al.*, 1992. *Cell* 69:551-558); p62 binding to proteins with 5H3 domains, p62 binding to RNA (see *e.g.*, Wang, *et al.*, 1995. *J. Biol. Chem.* 270:2010-2013); interaction with ubiquitin (see *e.g.*, Vadlamudi, *et al.*, 1996. *J. Biol. Chem.* 271:20235-20237) or interaction with CSK (see *e.g.*, Neet & Hunter, 1995. *Mol. Cell. Biol.* 15:4908-4920).

Finally, the human hnRNP G protein binds RNA, thus, the complexes and proteins of the invention may be screened by measuring their affect on the levels of hnRNP G protein binding to RNA. The 53BP2-binding partners, β-tubulin and p62, have been implicated in the processes of cellular apoptosis, mRNA destabilization and ubiquitin-mediated proteolysis associated with neurodegenerative disease. The 53BP2•53B2-IP complexes, particularly the 53BP2β-tubulin and 53BP2•p62 complexes (and derivatives, analogs and fragments thereof), nucleic acids encoding the 53BP2 and 53BP2-IP genes; anti-53BP2•53BP2-IP complex antibodies and various other modulators of 53BP2•53BP2-IP complex activity may be tested for activity in treating or preventing neurodegenerative disease in *in vitro* and *in vivo* assays. In one embodiment of the present invention, a Therapeutic may be assayed for activity in treating or preventing neurodegenerative disease by contacting cultured cells that exhibit an indicator of a neurodegenerative disease including, but not limited to, hypersecretion of B-A4 peptide (see *e.g.*, Nakajima, *et al.*, 1985. *Proc. Natl. Acad. Sci. USA* 82:6325- 6329) *in vitro* with the Therapeutic, and comparing the level of said indicator in the cells contacted with the Therapeutic, with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing neurodegenerative disease. Specific examples of such cultured models for neurodegenerative disease include, but are not limited to: cultured rat endothelial cells from affected and nonaffected individuals (see *e.g.*, Maneiro, *et al.*, 1997. *Methods Find. Exp. Clin. Pharmacol.* 19:5-12); P19 murine embryonal carcinoma cells (see *e.g.*, Hung, *et al.*, 1992. *Proc Natl Acad. Sci USA* 89:9439-9443) and dissociated cell cultures of cholinergic neurons from nucleus basalis of Meynert (see *e.g.*, Nakajima, *et al.*, 1985. *Proc Natl Acad. Sci USA* 82:6325-6329).

In another embodiment of the present invention, a Therapeutic may also be assayed for activity in treating or preventing neurodegenerative disease by administering the Therapeutic to a test animal that exhibits symptom of a neurodegenerative disease including, but not limited to, cognitive dysfunction in behavior maze test, or that is predisposed to develop symptoms or a neurodegenerative disease, and measuring the change in said symptoms of the neurodegenerative disease after administration of said Therapeutic, wherein a reduction in the severity of the symptoms of the neurodegenerative or prevention of the symptoms of the neurodegenerative disease indicates that the Therapeutic has activity in treating or preventing neurodegenerative disease. Such a test animal can be any one of a number of animal models known in the art for neurodegenerative disease. These models, including those for Alzheimer's Disease and mental

retardation of trisomy 21, accurately mimic natural human autoimmune diseases. See *e.g.*, Farine, 1997. *Toxicol.* 119:29-35.

The 53BP2-binding partner, hnRNP G, has also been implicated in autoimmune disease. Accordingly, 53BP2•53BP2-IP complexes, particularly 53BP2•hnRNP G complexes (and derivatives, analogs and fragments thereof), nucleic acids encoding the 53BP2 and 53BP2-IP genes; anti-53BP2•53BP2-IP complex antibodies and various other modulators of the 53BP2•53BP2-IP complex activity, may be assayed for activity in treating or preventing neurodegenerative disease in *in vitro* and *in vivo* assays. In one embodiment, a Therapeutic of the present invention may be assayed for activity in treating or preventing autoimmune disease by contacting cultured cells that exhibit an indicator of an autoimmune reaction *in vitro*, such as but not limited to, secretion of chemokines (see e.g., Kunkel, *et al.*, 1996. *J. Leukocyte Biol.* 59:6-12) with the Therapeutic, and comparing the level of said indicator in the cells contacted with the Therapeutic with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing autoimmune disease.

In another embodiment, a Therapeutic of the present invention may be assayed for activity in treating or preventing autoimmune disease by administering said Therapeutic to a test animal exhibiting an autoimmune reaction or which test animal does not exhibit an autoimmune reaction and is subsequently challenged with an agent that elicits an autoimmune reaction, and measuring the change in the autoimmune reaction after the administration of said Therapeutic, wherein a reduction in said autoimmune reaction or a prevention of said autoimmune reaction indicates that the Therapeutic has activity in treating or preventing an autoimmune disease. A number of animal models of autoimmune disease are known within the art. These models, including those for arthritis, systemic lupus erythematosus, diabetes, thyroiditis, encephalitis and the like, accurately mimic natural human autoimmune diseases. See *e.g.*, Farine, 1997. *Toxicol.* 119:29-35.

(14) SCREENING FOR ANTAGONISTS AND AGONISTS OF 53BP2•53BP2-IP COMPLEX AND 53BP2:IP-1, 53BP2:IP-2, AND 53BP2:IP-3

53BP2•53BP2-IP complexes, 53BP2:IP-1, 53BP2:IP-2 53BP2:IP-3 (and derivatives, fragments and analogs thereof), as well as nucleic acids encoding 53BP2 and 53BP2-IPs and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 (as well as derivatives, fragments and analogs thereof) may be utilized to screen for compounds which bind to 53BP2•53BP2-IP complexes and

53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 nucleic acids, proteins or derivatives, and thus have potential use as agonists or antagonists of 53BP2•53BP2-IP complex or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein function. The present invention thus provides assays to detect molecules that specifically bind to 53BP2 and 53BP2-IP, and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 nucleic acids, proteins or derivatives.

For example, recombinant cells expressing both 53BP2 and 53BP2-IP nucleic acids or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 nucleic acids may be used to recombinantly produce the complexes or proteins in these assays, to screen for molecules that bind or interfere with 53BP2•53BP2-IP complexes or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 function. In preferred embodiments, polypeptide analogs that have superior stability (but retain the ability to form 53BP2•53BP2-IP complexes), for example, 53BP2 and 53BP2-IPs which have been modified to be resistant to proteolytic degradation in the binding assay buffers, or to be resistant to oxidative degradation are used to screen for modulators (*e.g.*, molecules generated by substitution of amino acids at proteolytic cleavage sites, the use of chemically derivatized amino acids at proteolytic susceptible sites, and replacement of amino acid residues subject to oxidation, such as methionine and cysteine). Molecules (*e.g.*, putative binding partners of a 53BP2•53BP2-IP complex or of 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3) are contacted with the 53BP2•53BP2-IP complex or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 proteins (or fragment thereof) under conditions conducive to binding, and then molecules that specifically bind to 53BP2•53BP2-IP complexes or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 proteins are identified. Similar methods may be utilized to screen for molecules which bind to 53BP2•53BP2-IP or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 nucleic acids or derivatives thereof.

A particular aspect of the invention relates to identifying molecules that inhibit or promote formation or degradation of a 53BP2•53BP2-IP complex (*e.g.*, using the method described for screening inhibitors using the modified yeast two hybrid assay described *infra* and in U.S. Patent Application Nos. 08/663,824 and 08/874,825, both entitled "Identification and Comparison of Protein-Protein Interactions that Occur in Populations and Identification of Inhibitors of These Interactions,", by Nandabalan, *et al.*, which are incorporated by reference herein in their entireties. In one embodiment of the present invention, a molecule which modulates activity of 53BP2 or a protein selected from the group consisting of β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 (or a complex of 53BP2 and said protein) is identified by contacting one or more candidate molecules with 53BP2 in the presence of said protein, and measuring the amount of complex that forms between 53BP2 and said protein,

wherein an increase or decrease in the amount of complex that forms relative to the amount that forms in the absence of the candidate molecules indicates that the molecules modulate the activity of 53BP2 or said protein or said complex of 53BP2 and said protein. In preferred embodiments, the modulators are identified by administering the candidate molecules to a transgenic, non-human animal expressing both 53BP2 and a 53BP2-IP from promoters which are not the native 53BP2 or the native 53BP2-IP promoters, and more preferably where the candidate molecules are also recombinantly expressed in the transgenic, non-human animal. Alternatively, the method for identifying such modulators can be carried out *in vitro*, preferably with purified 53BP2, purified 53BP2-IP and purified candidate molecules.

Methodologies which can be used to carry out the foregoing are well-known within the art. Agents to be screened can be provided as mixtures of a limited number of specified compounds, or as compound libraries, peptide libraries and the like. Agents to be screened may also include all forms of antisera, antisense nucleic acids, etc. which possess the ability to modulate 53BP2•53BP2-IP complex activity or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 activity. By way of example, and not of limitation, diversity libraries (*e.g.*, random or combinatorial peptide or non-peptide libraries) may be screened for molecules which specifically bind to a 53BP2•53BP2-IP complex or 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein. Many libraries are known within the art and include, but are not limited to, chemically-synthesized libraries, recombinant (*e.g.*, phage display libraries) and *in vitro* translation-based libraries. Screening the libraries may be accomplished by any of a variety of commonly known methodologies. In a specific embodiment, screening may be performed by contacting the library members with a 53BP2•53BP2-IP complex or a 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 protein (or nucleic acid or derivative) which has been immobilized on a solid phase and harvesting those library members which bind to the protein (or nucleic acid or derivative). Examples of such screening methods, termed "panning" techniques are described by way of example, but not of limitation, in Fowlkes, *et al.*, 1992. *BioTechniques* 13:422-427; PCT Publication No. WO 94/18318.

In yet another specific embodiment of the present invention, fragments and/or analogs of 53BP2 or a 53BP2-IP, especially peptidomimetics, may be screened for activity as competitive or non-competitive inhibitors of 53BP2•53BP2-IP complex formation, and as a result concomitantly inhibit 53BP2•53BP2-IP complex activity. In a preferred embodiment, molecules which bind to 53BP2•53BP2-IP complexes and 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 proteins may be screened for using the modified, yeast two hybrid system described herein *infra*. In one embodiment, agents which modulate (*i.e.*, inhibit, antagonize or agonize) 53BP2•53BP2-

IP complex activity may be screened using a binding inhibition assay, wherein agents are screened for their ability to inhibit formation of a 53BP2•53BP2-IP complex under physiological binding conditions, in which 53BP2•53BP2-IP complex formation occurs in the absence of the agent to be tested. Agents which interfere with the formation of 53BP2•53BP2-IP complexes are identified as antagonists of said complex formation.

Methods for screening may also involve labeling the complex proteins with: (*i*) radioligands (*e.g.*, ^{125}I or ^{3}H); (*ii*) magnetic ligands (*e.g.*, paramagnetic beads covalently attached to photobiotin acetate); (*iii*) florescent ligands (*e.g.*, fluorescein or rhodamine) or (*iv*) enzyme ligands (*e.g.*, luciferase or β-galactosidase). The reactants which bind in an aqueous solution (*i.e.*, under physiological conditions) may then be isolated by one of many techniques known in the art including, but not limited to, co-immunoprecipitation of the labeled moiety using antisera against the unlabeled binding partner (or labeled binding partner with a distinguishable marker from that used on the labeled moiety) protein, immunoaffinity chromatography, size exclusion chromatography, and gradient density centrifugation. In a preferred embodiment, one binding partner is a small fragment or peptidomimetic that is not retained by a commercially available filter. Upon binding, the labeled species is then unable to pass through the filter, providing for a simple assay of complex formation. Methods commonly known in the art are used to label at least one of the members of the 53BP2•53BP2-IP complex. Suitable labeling includes, but is not limited to: (*i*) radiolabeling by incorporation of radiolabeled amino acids (*e.g.*, ^{3}H-leucine or ^{35}S-methionine, radiolabeling by post-translational iodination with ^{125}I or ^{131}I using the chloramine-T method, Bolton-Hunter reagents, etc., or labeling with ^{32}P using phosphorylase and inorganic radiolabeled phosphorous; (*ii*) biotin labeling with photobiotin-acetate and (*iv*) UV exposure, and the like. In cases where one of the members of the 53BP2•53BP2-IP complex is immobilized on a solid-support, the free species is labeled. Where neither of the interacting species is immobilized, each can be labeled with a distinguishable marker such that isolation of both moieties can be followed to provide for more accurate quantitation, and to distinguish the formation of homomeric from heteromeric complexes. Methods which utilize accessory proteins that bind to one of the modified interactants to improve the sensitivity of detection, increase the stability of the complex, etc. are also provided herein.

Typical binding conditions are, for example, but not by way of limitation, in an aqueous salt solution of 10-250 mM NaCl, 5-50 mM Tris-HCl, pH 5.8, 0.5% Triton X-100 or other detergent which improves specificity of interaction. Metal chelators and/or divalent cations may

be added to improve binding and/or reduce proteolysis. Reaction temperatures may include 4, 10, 15, 22, 25, 35, or 42 degrees Celsius, and time of incubation is typically at least 15 seconds, but longer times are preferred to allow binding equilibrium to occur. Particular 53BP2•53BP2-IP complexes can be assayed using routine protein binding assays to determine optimal binding conditions for reproducible binding. The physical parameters of complex formation may be analyzed by quantitation of complex formation using assay methods specific for the label used (*e.g.*, liquid scintillation counting for radioactivity detection, enzyme activity measurements for enzyme label, etc). The reaction results are then analyzed utilizing Scatchard analysis, Hill analysis, and other methods well-known within the arts. See *e.g.*, *Proteins, Structures, and Molecular Principles* 1984. (W.H. Freeman and Company, New York, NY).

In a second common approach to binding assays, one of the binding species is immobilized on a solid support (*e.g.*, on a filter, in a microtiter plate well, in a test tube, to a chromatography matrix, and the like) either covalently or non-covalently. Proteins may be covalently immobilized using any method well known in the art including, but not limited to, linkage to a cyanogen bromide-derivatized substrate such as CNBr-Sepahrose 4B. See *e.g.*, Kadonaga & Tjian, 1986. *Proc. Natl. Acad. Sci. USA* 83:5889-5893. Where needed, the use of spacers can reduce steric hindrance from the substrate. Non-covalent attachment of proteins to a substrate include, but are not limited to, attachment of a protein to a charged surface, binding with specific antibodies, binding to a third unrelated IP, and the like. In one specific embodiment of the present invention, immobilized 53BP2 is used to assay for binding with a radioactively-labeled 53BP2-IP in the presence and absence of a compound to be tested for its ability to modulate 53BP2•53BP2-IP complex formation. The binding partners are allowed to bind under aqueous, or physiological, conditions (*i.e.*, the conditions under which the original interaction was detected). Conversely, in another embodiment, the 53BP2-IP is immobilized and contacted with the labeled 53BP2 protein (or derivative thereof) under binding conditions.

Assays of agents (including cell extracts or library pool) for competition for binding of one member of a 53BP2•53BP2-IP complex (or derivatives thereof) with the other member of the 53BP2•53BP2-IP complex, are disclosed herein to screen for competitors of 53BP2•53BP2-IP complex formation. In specific embodiments, blocking agents to inhibit non-specific binding of reagents to other protein components, or absorptive losses of reagents to plastics, immobilization matrices, etc., are included in the assay mixture. Blocking agents include, but are not restricted to, bovine serum albumin (BSA), β-casein, nonfat dried milk, Denhardt's reagent, Ficoll, polyvinylpyrolidine, non-ionic detergents(*e.g.*, NP40, Triton X-100, Tween 20, Tween 80,

and the like), ionic detergents (*e.g.*, SDS, LDS, etc.), polyethyleneglycol, etc. Appropriate blocking agent concentrations allow 53BP2•53BP2-IP complex formation. After binding is performed, unbound, labeled protein is removed in the supernatant, and the immobilized protein with any bound, labeled protein is washed extensively. The amount of label bound is then quantitatively determined using standard methods in the art to detect the label as described *supra*.

(15) ASSAYS FOR PROTEINS-PROTEIN INTERACTIONS

One aspect of the present invention provides methods for assaying and screening fragments, derivatives and analogs of derivatives, analogs and fragments of 53BP2-interacting proteins (for binding to 53BP2 peptides). Derivatives, analogs and fragments of 53BP2-IPs which interact with the 53BP2 protein may be identified by means of a yeast two hybrid assay system. See Fields & Song, 1989. *Nature* 340: 245-246; U.S. Patent No. 5,283,173. or, more preferably, an improvement thereof as described in U.S. Patent Application Nos. 08/663,824 and 08/874,825, both entitled "Identification and Comparison of Protein-Protein Interactions that Occur in Populations and Identification of Inhibitors of These Interactions," by Nandabalan, *et al.*, which are incorporated by reference herein in their entireties. Because the interactions are screened for in yeast, the intermolecular protein interactions detected in this system generally occur under physiological conditions that mimic the conditions in mammalian cells. See *e.g.*, Chien, *et al.*, 1991. *Proc. Natl. Acad. Sci. USA* 88:9578-9581.

Identification of interacting proteins by the improved yeast two hybrid system is based upon the detection of the expression of a reporter gene ("Reporter Gene"), the transcription of which is dependent upon the reconstitution of a transcriptional regulator by the interaction of two proteins, each fused to one half of the transcriptional regulator. The "bait" (53BP2 or a derivative or analog) and "prey" (proteins to be tested for ability to interact with the bait) proteins are expressed as fusion proteins to a DNA binding domain, and to a transcriptional regulatory domain, respectively, or *vice versa*. In various specific embodiments of the present invention, the prey has a complexity of at least 50, 100, 500, 1,000, 5,000, 10,000, or 50,000, or has a complexity in the range of 25 to 100,000, 100 to 100,000, 50,000 to 100,000, or 10,000 to 500,000. For example, the prey population can be one or more nucleic acids encoding mutants of a 53BP2-IP (*e.g.*, as generated by site-directed mutagenesis or another method of making mutations in a nucleotide sequence). Preferably, the prey populations are proteins encoded by DNA (*e.g.*, cDNA, genomic DNA or synthetically generated DNA). For example, the populations can be expressed from chimeric genes comprising cDNA sequences from an

non-characterized sample of a population of cDNA from mammalian RNA. Preferably, the prey population are proteins encoded by DNA (*e.g.*, cDNA, genomic DNA or synthetically generated DNA). In a specific embodiment, recombinant biological libraries expressing random peptides may be used as the source of prey nucleic acids. In another specific embodiment, the invention provides methods for screening for inhibitors of the interacting proteins identified herein. In brief, the protein-protein interaction assay may be performed as described herein, except that it is done in the presence of one or more candidate molecules. An increase or decrease in Reporter Gene activity relative to that present when the one or more candidate molecules are absent indicates that the candidate molecule has an effect on the interacting pair. In a preferred embodiment, inhibition of the interaction is selected for (*i.e.*, inhibition of the interaction is necessary for the cells to survive), for example, where the interaction activates the URA3 gene, causing yeast to die in medium containing the chemical 5-fluoroorotic acid. See *e.g.*, Rothstein, 1983. *Meth. Enzymol.* 101:167-180. The identification of inhibitors of such interactions can also be accomplished, for example, but not by way of limitation, using competitive inhibitor assays, as described *supra*.

In general, proteins of the bait and prey populations are provided as fusion (chimeric) proteins (preferably by recombinant expression of a chimeric coding sequence) containing each protein contiguous to a pre-selected sequence. For one population, the pre-selected sequence is a DNA binding domain. The DNA binding domain may be any DNA binding domain, so long as it specifically recognizes a DNA sequence within a promoter. For example, the DNA binding domain is of a transcriptional activator or inhibitor. For the other population, the pre-selected sequence is an activator or inhibitor domain of a transcriptional activator or inhibitor, respectively. The regulatory domain alone (not as a fusion to a protein sequence) and the DNA-binding domain alone (not as a fusion to a protein sequence) preferably do not detectably interact, so as to avoid false-positives in the assay results.

The assay system of the present invention further includes a Reporter Gene operably-linked to a promoter which possesses a binding site for the DNA binding domain of the transcriptional activator (or inhibitor). Accordingly, in the embodiment of the invention, binding of a 53BP2 fusion protein to a prey fusion protein leads to reconstitution of a transcriptional activator (or inhibitor) which activates (or inhibits) expression of the Reporter Gene. The activation of transcription of the Reporter Gene occurs intracellularly (*e.g.*, in prokaryotic or eukaryotic cells, preferably in cell culture). The promoter which is operably-linked to the Reporter Gene nucleotide sequence may be a native or non-native promoter of the nucleotide

sequence, and the DNA binding site(s) which are recognized by the DNA binding domain portion of the fusion protein can be native to the promoter (if the promoter normally contains such binding site(s)) or non-native. Thus, for example, one or more tandem copies (*e.g.*, 4 or 5 copies) of the appropriate DNA binding site can be introduced upstream of the TATA box in the desired promoter (*e.g.*, in the area of position -100 to -400). In a preferred embodiment, 4 or 5 tandem copies of the 17 bp UAS (GAL4 DNA binding site) are introduced upstream of the TATA box in the desired promoter, which is upstream of the desired coding sequence for a selectable or detectable marker. In another preferred embodiment, the GAL1-b promoter is operably fused to the desired nucleotide sequence, the GAL1-b promoter already contains binding sites for GAL4.

Alternatively, the transcriptional activation binding site of the desired gene(s) may be deleted and replaced with GAL4 binding sites. See *e.g.*, Bartel, *et al.*, 1993. *BioTechniques* 14(6):920-924. The Reporter Gene preferably contains the sequence encoding a detectable or selectable marker the expression of which is regulated by the transcriptional activator, such that the marker is either turned on or off in the cell in response to the presence of a specific interaction. Preferably, the assay is carried out in the absence of background levels of the transcriptional activator (*e.g.*, in a cell that is mutant or otherwise lacking in the transcriptional activator). In one embodiment, more than one Reporter Gene is used to detect transcriptional activation (*e.g.*, one Reporter Gene encoding a detectable marker and one or more Reporter Genes encoding different selectable markers). The detectable marker may be any molecule that can give rise to a detectable signal, such as a fluorescent protein or a protein that can be readily visualized or that is recognizable by a specific antibody. The selectable marker may be any protein molecule that confers ability to grow under conditions that do not support the growth of cells not expressing the selectable marker (*e.g.*, the selectable marker is an enzyme that provides an essential nutrient and the cell in which the interaction assay occurs is deficient in the enzyme and the selection medium lacks such nutrient). The Reporter Gene may either be under the control of the native promoter that naturally contains a binding site for the DNA binding protein, or under the control of a heterologous or synthetic promoter. The activation domain and DNA binding domain used in the assay may be derived from a wide variety of transcriptional activator proteins, so long as these transcriptional activators have separable binding and transcriptional activation domains. For example, the GAL4 protein of *S. cerevisiae*; the GCN4 protein of *S. cerevisiae* (see *e.g.*, Hope & Struhb, 1986. *Cell* 46:885-894); the ARD1 protein of *S. cerevisiae* (see *e.g.*, Thukral, *et al.*, 1989. *Mol. Cell. Biol.* 9:2360-2369) and the human estrogen receptor

(see *e.g.*, Kumar, *et al.*, 1987. *Cell* 51:941-951) all possess separable DNA binding and activation domains.

Moreover, the DNA binding domain and activation domain that are employed in the fusion proteins need not be from the same transcriptional activator. In a specific embodiment of the present invention, a GAL4 or LEXA DNA binding domain is employed. In another specific embodiment, a GAL4 or herpes simplex virus VP16 (see *e.g.*, Triezenberg, *et al.*, 1988. *Genes Dev.* 2:730-742) activation domain is employed. In another specific embodiment, amino acid residues 1-147 of GAL4 (see *e.g.*, Ma, *et al.*, 1987. *Cell* 48:847-853) comprises the DNA binding domain; whereas amino acid residues 411-455 of VP16 (see *e.g.*, Triezenberg, *et al.*, 1988. *Genes Dev.* 2:730-742) comprises the activation domain. In a preferred embodiment of the present invention, the yeast transcription factor GAL4 is reconstituted by the protein-protein interaction and the host strain is mutant for GAL4. In another preferred embodiment, the DNA-binding domain is Acel and/or the activation domain is Acel, the DNA binding and activation domains of the Acel protein, respectively. Acel is a yeast protein that activates transcription from the CURL operon in the presence of divalent copper. CUP1 encodes metallothionein, which chelates copper, and the expression of CUP1 protein allows growth in the presence of copper, which is otherwise toxic to the host cells. The Reporter Gene may also be a CUP1-lacZ fusion which expresses the enzyme β-galactosidase (which is detectable by routine chromogenic assay) upon binding of a reconstituted AcelN transcriptional activator. See *e.g.*, Chaudhuri, *et al.*, 1995. *FEBS Letters* 357:221-226.

In another specific embodiment, the DNA binding domain of the human estrogen receptor is used, with a Reporter Gene driven by one or three estrogen receptor response elements (see *e.g.*, Le Douarin, *et al.*, 1995. *Nucl. Acids. Res.* 23:876-878). The DNA binding domain and the transcription activator/inhibitor domain each preferably has a nuclear localization signal (see *e.g.*, Ylikomi, *et al.*, 1992. *EMBO J.* 11:3681-3694) functional in the cell in which the fusion proteins are to be expressed. To facilitate the subsequent isolation of the encoded proteins, the fusion constructs can further contain sequences encoding "affinity tags" (*e.g.*, glutathione-S-transferase, maltose-binding protein or an epitope of an available antibody) for affinity purification. See *e.g.*, Allen, *et al.*, 1995. *TIBS* 20:511-516. In yet another embodiment, the fusion constructs further comprise bacterial promoter sequences for recombinant production of the fusion protein in bacterial cells. See *e.g.*, Allen, *et al.*, 1995. *TIBS* 20:511-516.

The host cell in which the interaction assay occurs can be any cell, prokaryotic or eukaryotic, in which transcription of the Reporter Gene can occur and be detected including, but

not limited to, mammalian (*e.g.*, monkey, chicken, mouse, rat, human, bovine), bacteria, insect cells, or, preferably, a yeast cell. Expression constructs encoding and capable of expressing the binding domain fusion proteins, the transcriptional activation domain fusion proteins, and the Reporter Gene product(s) are provided within the host cell, by mating of cells containing the expression constructs, or by cell fusion, transformation, electroporation, microinjection, etc. In a specific embodiment of the present invention in which the assay is carried out in mammalian cells (*e.g.*, hamster cells), the DNA binding domain is the GAL4 DNA binding domain, the activation domain is the herpes simplex virus VP16 transcriptional activation domain, and the Reporter Gene contains the desired coding sequence operably-linked to a minimal promoter element from the adenovirus E1B gene driven by several GAL4 DNA binding sites (see *e.g.*, Fearon, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89:7958-7962). The host cell used should not express an endogenous transcription factor that binds to the same DNA site as that recognized by the DNA binding domain fusion population. Also, preferably, the host cell is mutant or otherwise lacking in an endogenous, functional form of the Reporter Gene(s) used in the assay. Various vectors and host strains for expression of the two fusion protein populations in yeast are known and may be utilized. See *e.g.*, U.S. Patent No. 5,1468,614; Fields & Sternglanz, 1994. *TIG* 10:286-292. By way of example but not limitation, yeast strains or derivative strains made therefrom, which may be utilized include: ITIOS, N106R, N1051, N106R1, and YULH, although numerous other strains commonly-known and available within the art can be used.

If not already lacking in endogenous Reporter Gene activity, cells mutant in the Reporter Gene may be selected by known methods, or the cells can be made mutant in the target Reporter Gene by known gene-disruption methods prior to introducing the Reporter Gene. See *e.g.*, Rothstein, 1983. *Meth. Enzymol.* 101:202-211. In a specific embodiment of the present invention, plasmids encoding the different fusion protein populations can be both introduced into a single host cell (*e.g.*, a haploid yeast cell) containing one or more Reporter Genes, by co-transformation, to conduct the assay for protein-protein interactions. Or, preferably, the two fusion protein populations are introduced into a single cell either by mating (*e.g.*, of yeast cells) or by cell fusions (*e.g.*, of mammalian cells). In a mating type assay, conjugation of haploid yeast cells of opposite mating type that have been transformed with a binding domain fusion expression construct (preferably a plasmid) and an activation (or inhibitor) domain fusion expression construct (preferably a plasmid), respectively, delivers both constructs into the same diploid cell.

The mating type of a yeast strain may be manipulated by transformation with the HO gene. See *e.g.*, Herskowitz & Jensen, 1991. *Meth. Enzymol.* 194:132-146. In a preferred embodiment, a yeast interaction mating assay is employed, using two different types of host cells, strain-types (a and alpha) of the yeast *Saccharomyces cerevisiae*. The host cell preferably contains at least two Reporter Genes, each with one or more binding sites for the DNA-binding domain (*e.g.*, of a transcriptional activator). The activator domain and DNA binding domain are each parts of chimeric proteins formed from the two respective populations of proteins. One set of host cells, for example the a strain cells, contains fusions of the library of nucleotide sequences with the DNA-binding domain of a transcriptional activator, such as GAL4. The hybrid proteins expressed in this set of host cells are capable of recognizing the DNA-binding site on the Reporter Gene. The second set of yeast host cells, for example alpha strain cells, contains nucleotide sequences encoding fusions of a library of DNA sequences fused to the activation domain of a transcriptional activator. In a preferred embodiment, the fusion protein constructs are introduced into the host cell as a set of plasmids. These plasmids are preferably capable of autonomous replication in a host yeast cell and preferably can also be propagated in *E. coli*. The plasmid contains a promoter directing the transcription of the DNA binding or activation domain fusion genes, and a transcriptional termination signal. The plasmid also preferably contains a selectable marker gene, permitting selection of cells containing the plasmid. The plasmid may be single-copy or multi-copy. Single-copy yeast plasmids that have the yeast centromere may also be used to express the activation and DNA binding domain fusions. See *e.g.*, Elledge, *et al.*, 1988. *Gene* 70:303-312. In another embodiment, the fusion constructs are introduced directly into the yeast chromosome via homologous recombination. The homologous recombination for these purposes is mediated through yeast sequences that are not essential for vegetative growth of yeast (*e.g.*, the MER2, MER, ZIPI, REC102 or ME14 genes).

Bacteriophage vectors may also be utilized to express the DNA binding domain and/or activation domain fusion proteins. Libraries can generally be prepared faster and more easily from bacteriophage vectors than from plasmid vectors. In a specific embodiment, the invention provides a method for the detection of one or more protein-protein interactions comprising: (*i*) recombinantly expressing 53BP2 (or a derivative or analog thereof) in a first population of yeast cells being of a first mating type and comprising a first fusion protein containing the 53BP2 sequence and a DNA binding domain, wherein said first population of yeast cells contains a first nucleotide sequence operably-linked to a promoter driven by one or more DNA binding sites recognized by said DNA binding domain such that an interaction of said first fusion protein with

a second fusion protein, said second fusion protein comprising a transcriptional activation domain, results in increased transcription of said first nucleotide sequence; (*ii*) negatively-selecting to eliminate those yeast cells in said first population in which said increased transcription of said first nucleotide sequence occurs in the absence of said second fusion protein; (*iii*) recombinantly-expressing in a second population of yeast cells of a second mating type different from said first mating type, a plurality of said second fusion proteins, each second fusion protein comprising a sequence of a fragment, derivative or analog of a 53BP2-IP and an activation domain of a transcriptional activator, in which the activation domain is the same in each said second fusion protein; (*iv*) mating said first population of yeast cells with said second population of yeast cells to form a third population of diploid yeast cells, wherein said third population of diploid yeast cells contains a second nucleotide sequence operably-linked to a promoter driven by a DNA binding site recognized by said DNA binding domain such that an interaction of a first fusion protein with a second fusion protein results in increased transcription of said second nucleotide sequence, in which the first and second nucleotide sequences can be the same or different and (*v*) detecting said increased transcription of said first and/or second nucleotide sequence, thereby detecting an interaction between a first fusion protein and a second fusion protein.

In a preferred embodiment of the present invention, the bait 53BP2 sequence and the prey library of chimeric genes are combined by mating the two yeast strains on solid media for a period of approximately 6-8 hours. In a less preferred embodiment, the mating is performed in liquid media. The resulting diploids possess both types of chimeric genes (*i.e.*, the DNA-binding domain fusion and the activation domain fusion). Preferred Reporter Genes include, but are not limited to, URA3, HIS3 and/or the lacZ genes (see *e.g.*, Rose & Botstein, 1983. *Meth. Enzymol.* 101:167-180) operably-linked to GAL4 DNA-binding domain recognition elements. Other reporter genes comprise the functional coding sequences for, but not limited to, Green Fluorescent Protein (GFP; see *e.g.*, Cubitt, *et al.*, 1995. *Trends Biochem. Sci.* 20:448-455), luciferase, LEU2, LYS2, ADE2, TRP1, CANi, CYH2, GUS, CUP1 or chloramphenicol acetyl transferase (CAT). Expression of LEU2, LYS2, ADE2 and TRP1 are detected by growth in a specific, defined media; GUS and CAT may be monitored by well-known enzyme assays and CANi and CYH2 are detected by selection in the presence of canavanine and cycloheximide. With respect to GFP, the natural fluorescence of the protein is detected.

In a specific embodiment of the present invention, transcription of the Reporter Gene is detected by a linked replication assay. For example, expression of SV40 large T antigen is under

the control of the ElB promoter responsive to GAL4 binding sites. See *e.g.*, Vasavada, *et al.*, 1991. *Proc. Natl. Acad. Sci. USA* 88:10686-10690. The replication of a plasmid containing the SV40 origin of replication, indicates the reconstruction of the GAL4 protein and a protein-protein interaction. Alternatively, a polyoma virus replicon can be employed. See *e.g.*, Vasavada, *et al.*, 1991. *Proc. Natl. Acad. Sci. USA* 88:10686-10690. In another embodiment, the expression of Reporter Genes that encode proteins can be detected by immunoassay (*i.e.*, by detecting the immunospecific binding of an antibody to such protein, which antibody can be labeled, or alternatively, which antibody can be incubated with a labeled binding partner to the antibody, so as to yield a detectable signal). Alam & Cook, (1990. *Anal. Biochem.* 188:245-254) disclose non-limiting examples of detectable marker genes that can be operably linked to a transcriptional regulatory region responsive to a reconstituted transcriptional activator, and thus used as Reporter Genes.

The activation of Reporter Genes like URA3 or HIS3 enables the cells to grow in the absence of uracil or histidine, respectively, and hence serves as a selectable marker. Thus, after mating, the cells exhibiting protein-protein interactions are selected by the ability to grow in media lacking a nutritional component, such as uracil or histidine, respectively (referred to as -URA (minus ElBA) and -HIS (minus HIS) medium, respectively). The -HIS medium preferably contains 3-amino-l,2,4-triazole (3-AT), which is a competitive inhibitor of the HIS3 gene product and thus requires higher levels of transcription in the selection. See *e.g.*, Durfee, *et al.*, 1993. *Genes Dev.* 7:555-569. Similarly, 6-azauracil, which is an inhibitor of the URA3 gene product, may also be included in -URA medium. See *e.g.*, Le Douarin, *et al.*, 1995. *Nucl. Acids Res.* 23:876-878. URA3 gene activity can also be detected and/or measured by determining the activity of its gene product, orotidine monophosphate decarboxylase. See *e.g.*, Pierrat, *et al.*, 1992. *Gene* 119:237-245.

In other embodiments of the present invention, the activities of the reporter genes like lacZ or GFP are monitored by measuring a detectable signal (*e.g.*, fluorescent or chromogenic) which results from the activation of these Reporter Genes. For example, lacZ transcription can be monitored by incubation in the presence of a chromogenic substrate, such as X-gal (5-bromo-4-chloro-3-indolyl-a-D-galactoside), for its encoded enzyme, β-galactosidase. The pool of all interacting proteins isolated by this manner from mating the 53BP2 sequence product and the library identifies the "53BP2 interactive population." In a preferred embodiment of the present invention, false positives arising from transcriptional activation by the DNA binding domain fusion proteins in the absence of a transcriptional activator domain fusion protein are prevented

or markedly reduced by negative selection for such activation within a host cell containing the DNA binding fusion population, prior to exposure to the activation domain fusion population. By way of example, and not of limitation, if such cell contains URA3 as a Reporter Gene, negative selection is carried out by incubating the cell in the presence of 5-fluoroorotic acid (5-FOA) which kills URA+ cells. See *e.g.*, Rothstein, 1983. *Meth. Enzymol.* 101:167-180. Hence, if the DNA-binding domain fusions by themselves activate transcription. the metabolism of 5-FOA will lead to cell death and the removal of self-activating DNA-binding domain hybrids. Negative selection involving the use of a selectable marker as a Reporter Gene and the presence in the cell medium of an agent toxic or growth inhibitory to the host cells in the absence of Reporter Gene transcription is preferred, since it allows a higher rate of processing than other methods. As will be apparent, negative selection can also be carried out on the activation domain fusion population prior to interaction with the DNA binding domain fusion population, by similar methods, either alone or in addition to negative selection of the DNA binding fusion population.

Negative selection may also be carried out on the recovered 53BP2•53BP2-IP complexes by known methods (see *e.g.*, Bartel, *et al.*, 1993. *BioTechniques* 14:920-924), although pre-negative selection (*i.e.*, prior to the interaction assay), as described *supra*, is preferred. For example, each plasmid encoding a protein (peptide or polypeptide) fused to the activation domain (one-half of a detected interacting pair) may be transformed back into the original screening strain, either alone or with a plasmid encoding only the DNA-binding domain, the DNA-binding domain fused to the detected interacting protein, or the DNA-binding domain fused to a protein that does not affect transcription or participate in the protein-protein interaction, a positive interaction detected with any plasmid other than that encoding the DNA-binding domain fusion to the detected interacting protein is deemed a false positive and eliminated from the screen.

In a preferred embodiment of the present invention, the 53BP2 plasmid population is transformed in a yeast strain of a first mating type (a or alpha), and the second plasmid population (containing the library of DNA sequences) is transformed in a yeast strain of different mating type. Both strains are preferably mutant for URA3 and HIS3, and contain HIS3, and optionally lacZ, as a Reporter Genes. The first set of yeast cells are positively-selected for the 53BP2 plasmids and are negatively-selected for false positives by incubation in medium lacking the selectable marker (*e.g.*, tryptophan) and containing 5-FOA. Yeast cells of the second mating type are then transformed with the second plasmid population, and are positively selected for the

presence of the plasmids containing the library of fusion proteins. The selected cells are subsequently pooled and mating is allowed to occur on a solid phase. The resulting diploid cells are then transferred to selective media that selects for the presence of each plasmid and for activation of Reporter Genes.

In another preferred embodiment of the present invention, after an interactive population is obtained, the DNA sequences encoding the pairs of interactive proteins are isolated by a method wherein either the DNA-binding domain hybrids or the activation domain hybrids are amplified, in separate respective reactions. Preferably, the amplification is carried out by polymerase chain reaction (PCR; see *e.g.*, U.S. Patent Nos. 4,683,202, 4,683,195 and 4,889,818 and Gyllenstein, *et al.*, 1988. *Proc. Natl. Acad. Sci. USA* 85:7652-7656) using pairs of oligonucleotide primers specific for either the DNA-binding domain hybrids or the activation domain hybrids. This PCR amplification reaction may also be performed on pooled cells expressing interacting protein pairs, preferably pooled arrays of interactants. Other amplification methods which are well-known within the art may also be utilized in the practice of the present invention including, but not limited to: ligase chain reaction, Q/3 replicase, or various other methods enumerated in Kricka, *et al.*, 1995. *Molecular Probing, Blotting, and Sequencing* (Academic Press, New York, NY).

The plasmids encoding the DNA-binding domain hybrid and the activation domain hybrid proteins can also be isolated and cloned by any of the methods well-known within the art. For example, but not by way of limitation, if a shuttle (yeast to *E. coli*) vector is used to express the fusion proteins, the genes can be recovered by transforming the yeast DNA into *E. coli* and recovering the plasmids from *E. coli*. See *e.g.*, Hoffman, *et al.*, 1987. *Gene* 57:267-272. Alternatively, the yeast vector can be isolated, and the insert encoding the fusion protein subcloned into a bacterial expression vector, for growth of the plasmid in E. coli.

(16) PHARMACEUTICAL COMPOSITIONS AND THERAPEUTIC/PROPHYLACTIC ADMINISTRATION

The present invention discloses methods of treatment (and prophylaxis) by administration to a subject of an effective amount of a Therapeutic. In a preferred embodiment, the Therapeutic is substantially purified. The subject is preferably an animal including, but not limited to, animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human. In another specific embodiment, a non-human mammal is the subject. Formulations and methods of administration that can be employed when the Therapeutic

comprises a nucleic acid are described *supra*, and additional appropriate formulations and routes of administration may be selected from among those described herein below.

Various delivery systems are known and can be used to administer a Therapeutic of the invention including, but not limited to: encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the Therapeutic, receptor-mediated endocytosis (see *e.g.*, Wu & Wu, 1987. *J. Biol. Chem.* 262:4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, and the like. Methods of introduction include, but are not limited to: intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration may be either systemic or local. Furthermore, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection, intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir (*e.g.*, an Ommaya reservoir). Pulmonary administration may also be employed (*e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent).

In another specific embodiment of the present invention, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment, this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application (*e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers). In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue. In another embodiment, the Therapeutic may be delivered in a vesicle, in particular a liposome (see *e.g.*, Treat, *et al.*, In: *Liposomes in the Therapy of Infectious Disease and Cancer* (Liss, New York, NY).

In yet another embodiment, the Therapeutic may be delivered in a controlled release system. In one specific embodiment, a pump may be utilized. See *e.g.*, Sefton, 1987. *CRC Crit. Ref. Biomed. Eng.* 14:201. In another embodiment, polymeric materials can be used (see *e.g.*, *Medical Applications of Controlled Release* 1984. (CRC Pres., Boca Raton, FL). In yet another

embodiment, a controlled release system can be placed in proximity of the therapeutic target (*e.g.*, the brain), thus requiring only a fraction of the systemic dose. In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid may be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, (*e.g.*, by use of a retroviral vector; see U.S. Patent No. 4,980,286), or by direct injection; or by use of microparticle bombardment (*e.g.*, a gene gun, Biolistic, DuPont), or coating with lipids or cell-surface receptors or transfecting agents; or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot, *et al.*, 1991. *Proc. Natl. Acad. Sci. USA* 88:1864-1868), and the like. Alternatively, a nucleic acid Therapeutic can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable," as used herein, means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly, in humans. The term "carrier," as used herein, refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose,

magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin (1965).

Such compositions will contain a therapeutically effective amount of the Therapeutic (preferably in a substantially purified form) together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic (*e.g.*, lignocaine) to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachet indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration. The Therapeutics of the invention can be formulated as neutral or salt forms.

The amount of the Therapeutic of the present invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and may be determined in a quantitative manner by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 μg of active compound/kg of patient body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; whereas oral formulations preferably contain 10% to 95% active ingredient.

The present invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the

invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

SPECIFIC EXAMPLES

(A) IDENTIFICATION OF 53BP2•53BP2-IP COMPLEXES

A modified, improved yeast two hybrid system was utilized to identify protein interactions. Yeast is a eukaryote, and therefore any intermolecular protein interactions detected in this type of system demonstrate protein interactions that occur under physiological conditions. Expression vectors were constructed to encode two hybrid proteins. For a "forward" screen, one hybrid consisted of the DNA binding domain of the yeast transcriptional activator Gal4 fused to a portion of 53BP2. The other hybrid consisted of the Gal4 activator domain fused to "prey" protein sequences encoded by a mammalian cDNA library. In a "reverse" screen, the portion of 53BP2 was fused to the Gal4 activator domain, and the prey protein sequences of the mammalian cDNA library were fused to the DNA binding domain, but the assay was otherwise identically performed.

Each of the vectors was inserted into complementary (a and alpha) mating types of yeast using methods known in the art and mating was then carried out to express both vector constructs within the same yeast cells, thus allowing interaction to occur. Interaction between the bait and prey domains led to transcriptional activation of reporter genes containing cis-binding elements for Gal4. The Reporter Genes encoding the indicator protein (β-galactosidase) and metabolic markers for uracil and histidine auxotrophy, were included in specific fashion in one or the other of the yeast strains used in the mating. In this manner, yeast were selected for successful mating, expression of both fusion construct, and expression of 53BP2-IPs. Yeast clones which possessed interacting regions were picked and grown in individual wells of microtiter plates. The plasmids containing the 53BP2-IPs were then isolated and characterized. The prey cDNAs were obtained from a fetal brain cDNA library of 1.5×10^{10} independent isolates. The library was synthesized from Xho I-dT_{15}-primed fetal brain mRNA (from five male/female 19-22 week fetuses) which was directionally cloned into pBD-Gal4 (a yeast Gal4 DNA binding domain cloning vector including the TRYP gene for selection in yeast deficient in tryptophan biosynthesis). A reverse screen was used to test the interaction of prey cDNA products against an array of 22 bait

proteins, one of which was encoded by the 53BP2 nucleotide sequence of nucleotides 2866-3771 as depicted in Figure 1 [SEQ. ID NO:1], including amino acid residues 704-1005 of the 53BP2 amino acid sequence at the carboxyl-terminus of 53BP2, as depicted in Figure 1 [SEQ. ID NO:2]. The bait fragment was amplified from a λgt11 library (Clontech) by polymerase chain reaction (PCR) using the forward primer: 5'GGACTAGGCCGAGGTGGCCTCTCCAGGCCT TGATTATGAGCCTG3' [SEQ. ID NO:14] and the reverse primer: 5'GGACTAGGCCTCCTCGGCCCTACCTCTGCACTATGTCACTGATTTC3' [SEQ. ID NO:15], by standard techniques.

The resulting amplification fragment was cloned into the Sfi I site of the vector pACT-Sfi I, constructed by introducing an Sfi I-containing polylinker into the vector pACT2 (Clontech). This vector is a yeast activation domain cloning vector which contains the LEU2 gene for selection in yeast strains deficient in leucine biosynthesis. The bait was sequenced to confirm that PCR amplification reproduced an accurate copy of the 53BP2 sequence (see Figure 1). This analysis determined that, as predicted, the bait sequence encoded an interacting domain identical to the human 53BP2 beginning at amino acid residue 704 (see Figure 1). The bait was then transformed by a lithium acetate/polyethylene glycol transformation protocol (see Ito, *et al.*, 1983. *J. Bacteriol.* 153:163-168) into the yeast strain NIO6r (mating type a, ura3, his3, ade2, trpl, leu2, gal4, galsO, cyhr, Lys2::GAL1-^{11153}TATA^{11153} ura 3:GALlU- GALTATA-lacZ), while the prey sequences were transformed into the yeast strain YULH (mating type alpha, ura3, his3, lys2, Ade2, trpl, leu2, gal4, galBO, GALl-lacZ, GALl-URA3).

The two transformed populations were then mated using standard methods within the art. Briefly, cells were grown until mid-to-late log phase on media that selected for the presence of the appropriate plasmids. The two mating strains (alpha and a) were then diluted in YAPD media (see *e.g.*, Sherman, *et al.*, 1991. *Getting Started with Yeast, Vol. 194* (Academic Press, New York, NY), filtered onto nitrocellulose membranes, and incubated at 30°C for 6-8 hours. The cells were then transferred to media selective for the desired diploids (*i.e.*, yeast possessing Reporter Genes for β-galactosidase, uracil auxotrophy, and histidine auxotrophy, and expression of the vectors encoding the bait and prey.

The mating products were plated on SC (synthetic complete; see Kaiseret, *et al.*, 1994. *Methods in Genetics* (Cold Spring Harbor Press, New York, NY) media lacking adenine and lysine (to select for successful mating), leucine and tryptophan (to select for expression of genes encoded by both the bait and prey plasmids), and uracil and histidine (to select for protein interactions). This medium is hereinafter referred to as SCS medium, for SC Selective medium.

Selected clones were tested for expression of β-galactosidase to confirm the formation of a 53BP2•53BP2-IP interaction. Filter-lift β-galactosidase assays were performed as modified from the protocol of Breeden and Nasmyth, 1985. *Cold Spring Harbor Quant. Biol.* 50:643-650. Colonies were patched onto SCS plates, grown overnight, and replica-plated onto Whatman No. 1 filters. The filters were then assayed for β-galactosidase activity; wherein colonies which were "positive" turned a visible blue.

Cells in colonies positive for protein interaction contained a mixture of DNA-binding and activation-domain plasmids. These cells were individually plated, and regrown as single isolates in individual wells of 96-well microtiter plates. Approximately 10 μl of each isolate was then lysed, the inserts within the pACT2 and pASSPiI plasmids were amplified by PCR using primers specific for the flanking sequences of each vector, and approximately 200 amino-terminal nucleotides of each insert was determined using an ABI 377 sequenator. Comparison to known sequences was made using the "Blast" program publicly available through the National Center for Biotechnology Information (NCBI).

Two of the inserts were identified as β-tubulin, the others identified as p62, hnRNP G, and the insert encoding 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3. Specifically, the inserts contained nucleotides 830-1398 and 895-1398 of the coding sequence for β-tubulin, as depicted in Figure 2 [SEQ. ID NO:3]; nucleotides 929-1435 of the nucleotide sequence of p62, as depicted in Figure 3 [SEQ. ID NO:5], nucleotides 273-1322 of hnRNP G, as depicted in Figure 4 [SEQ. ID NO:7] and the sequence depicted in Figure 5 (encoding in-part 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3). The determined nucleic acid sequences and corresponding, inferred amino acid sequences of β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 are illustrated in Figures 2-4 and 9-13, respectively. A summary of the 53BP2 and 53BP2-IP interacting domains are shown in Figure 6.

(B) VERIFICATION OF THE SPECIFICITY OF THE 53BP2•β-TUBULIN, p62, hnRNP G AND 53BP2•IP-1, -2 and -3 INTERACTIONS

In order to ascertain the specificity of bait:prey interaction, two general tests were first performed. In the first test, Nl06 cells were created that express the individual plasmids encoding 53BP2, β-tubulin, p62, hnRNP G, and the sequences encoding 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3. These yeast cells were plated on SCS plates, grown overnight, and examined for growth. No growth was found for all five proteins, confirming that they were not "self-activating" proteins (*i.e.*, these proteins require interaction with a second protein domain

for a functional activation complex). In the second test, plasmids containing β-tubulin, p62, hnRNP G, and 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 inserts were transformed into strain YULH (mating type alpha) and mated with yeast strain N106 (mating type a) expressing proteins other than 53BP2. Promiscuous binders (*i.e.*, inserts able to bind with many other proteins in a non-specific fashion) would interact non-specifically with non-53BP2 domains, and would be discarded as non-specific interactants. 53BP2 was demonstrated to fail to interact with pRb (GenBank Acc. No. 1428419; Lee, *et al.*, 1987. *Nature* 329: 642-645); the *trk* oncogene (GenBank Acc. No. X03541; Martin-Zanca, *et al.*, 1986. *Nature* 319:743-748); EST M62042 (Adams, *et al.*, 1991. *Science* 252:1651-1656); Ral GDS (GenBank Acc. No. U14417; Hofer, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91:11089-11093) or E2F (GenBank Acc. No. X86096). In addition, β-tubulin, p62, hnRNP G, and the sequences encoding 53BP2:IP-1, 53BP2:IP-2 or 53BP2:IP-3 also did not interact with MDM2 15 (GenBank Acc. No. M92424); CAS (GenBank Acc. No. U33286: Brinkmann, *et al.*, 1995. *Proc. Natl. Acad. Sci. U.S.A.* 92:10427-10431) and PA9 (GenBank Acc. No. S82076; Yang, *et al.*, 1996. *Carcinogenesis* 17:563-567). Specifically, lack of growth for the p62 and β-tubulin interactions with MDM2 is depicted in Figure 7.

To recapitulate the detected interactions, and further demonstrate their specificity, the isolated bait plasmid for 53BP2, along with bait plasmids for MDM2 and human bait protein 1 (Bl) were utilized to transform yeast strain N106Rr (mating type a). The interacting domains from p62 and β-tubulin were transformed into strain YULH (mating type alpha). The transformants were re-amplified, and a mating performed to recapitulate the identified 53BP2•53BP2-IP complex formation. 53BP2 complexed specifically with β-tubulin and p62, but *not* with two human proteins, Hl and H2. As illustrated in Figure 7, the intersection of the 53BP2 row (bottom) with the β-tubulin and p62 columns indicates growth (*i.e.*, a positive interaction), but the intersection of the 53BP2 row with the columns for Hl and H2 indicates no growth (*i.e.*, no protein interaction). The known interaction between 53BP2 and PPl-α (see *e.g.*, Helps, *et al.*, 1995. *FEBS Letts.* 377:295-300) was confirmed, as shown in Figure 7, intersection of column 3, row 3. As previously described above, β-tubulin and p62 failed to interact with MDM2 and Bl. Mating of PPl-α and Bl confirmed an interaction previously found in our studies (Figure 7, and Nandabalan, *et al.*, 1997. unpublished).

(C) ASSEMBLY OF THE SEQUENCE ENCODING 53BP2:IP-1, 53BP2:IP-2 AND 53BP2:IP-3

One identified prey sequence was identical to EST R72810 (GenBank data base "dbest"). The database contained other EST sequences which were found to overlap the EST R72810 sequence. Hence, it was possible to find contiguous sequences to extend the nucleotide sequence both 3'- and 5'-termini of the EST R72810. The general procedure utilized is illustrated in Figure 8. The National Center for Biotechnology Information (NCBI) "Blast" Program was used to compare the EST R72810, and compared to all sequences in the non-redundant nucleotide data bases "NRDB," a compilation of GenBank + EMBL + DDBJ + PDB sequences (but no EST, STS, GSS, or HTGS sequences), as defined in the NCBI Blast program, "month," which includes all new or revised GenBank + EMBL + DDBJ + PDB sequences released within the last 30 days, and "dbest," a non-redundant database of GenBank + EMBL + DDBJ + EST divisions.

Sequences which aligned with 95% or greater identity at the nucleic acid level over their termini of at least 30 bases, were utilized if the alignment resulted in either a 5'-extension or 3'-extension of the EST R72810 sequence. Once this first assembly was complete, the extended sequence was again subject to the Blast comparison, so as to detect possible, new homologies to the added extensions. Specifically, the sequence was extended in both directions until new related sequences that allowed extension of the assembled sequence were no longer detected. The extended EST R72810 sequence is illustrated in Figure 9 and the nucleic acid sequence of the original EST R72810 sequence is shown in bold lettering.

For 5'-extension, a long overlap was found with EST C17385, the nucleotide sequence of which is denoted by bold underline (Fujiwara *et al.*, GenBank direct submission, Sept. 9.1996). For 3'-extension, overlap with EST AA464793 (Hillier, *et al.*, 1997. Wash Univ.-Merck EST Project), shown in boxed lettering, was detected. Additional overlap between EST AA464793 and EST AA479761 (Hillier, *et al.*, 1997. Wash Univ.-NCI Human EST Project), shown in bold, italic lettering, was utilized for further 3'-extension, to complete the EST assembly process. The complete assembled sequence of 915 nucleotides is shown in Figure 9. The assembled EST sequence was then subjected to a further 10 searches of both the NRDB and "month" nucleic acid data bases to detect possible homologies to known protein sequences which were not previously detected over the shorter-span of the original EST sequence. This step was necessary where significant homology was not detected during the EST assembly process to proteins in the NRDB and "month" data bases, as was the case with the assembled EST R72810. However, no

significant homologies to known proteins were detected for the extended EST R72810 utilizing this aforementioned analysis.

In addition, the sequence was analyzed by the NCBI program "ORF Finder," that performed translations in all three forward reading frames of the assembled DNA sequence (Figures 10 A-F). EST R72810 (Hillier, *et al.*, 1995. GenBank Direct Submission, June 2, 1995) was obtained from the directionally-cloned Soares breast library 2NbHBst. and thus the direction of 25 translation of the extended EST is known as 5' to 3'. Within the three translations, three possible open reading frames ("ORFs") were identified. Open reading frames greater than 60 amino acid residues in length following an initiator codon or an ORF with no initiator methionine encoded at the 5' end were determined to be possible protein products. and were submitted for Blast searching against the protein data base NRDB. a non-redundant compilation of GenBank CDS translations + PDB + SwissProt + PIR Swiss Prot sequences, and "month," which includes all new or revised GenBank CDS translation + PDB + Swiss Prot + PIR sequences released within the last 30 days.

Three possible protein candidates were identified by the hypothetical translation. The first candidate, encoded in Translation Frame #3 (Figure 10 F), encompasses an ORF from amino acid residue 1 (Arg) to amino acid residue 286 (Gln) which directly precedes an opal translational stop codon. This aforementioned translational frame has no initiator methionine codon (ATG), and hence must extend further 5' than the assembled sequence. The second protein candidate, encoded in Translation Frame #2 (Figure 10 D), extends from the initiator codon ATG at amino acid residue position 147 (Met) to a Gln residue, 69 amino acids downstream, which precedes an amber translational stop codon. The third protein candidate, encoded in Translation Frame #1 (Figure 10 B), extends from a Gly residue at amino acid residue position 1 (no initiation methionine codon was found in this reading frame) to a Gly amino acid residue located at position 33, which precedes a TAA stop codon. It should be noted that none of these aforementioned proteins displayed significant homology to any known protein at either the nucleic acid or amino acid levels.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, numerous modifications of the invention, in addition to those described herein, will become apparent to those individuals skilled within the relevant arts from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A purified complex of a 53BP2 protein and a 53BP2-IP protein, wherein the 53BP2-IP protein is not PP1-α or p53.

2. The purified complex of claim 1, wherein said 53BP2-IP protein is selected from the group consisting of β-tubulin protein, p62 protein, and hnRNP G protein.

3. The purified complex of claim 1, wherein said 53BP2-IP protein is a 53BP2:IP-l protein.

4. The purified complex of claim 1, wherein said 53BP2-IP protein is a 53BP2:IP-2 protein.

5. The purified complex of claim 1, wherein said 53BP2-IP protein is a 53BP2:IP-3 protein.

6. The purified complex of claim 1, in which said proteins are human proteins.

7. A purified complex selected from the group consisting of: a complex of a fragment of a 53BP2 protein and a full-length 53BP2-IP protein, a complex of a full-length 53BP2 protein and a fragment of a 53BP2-IP protein, and a complex of a fragment of a 53BP2 protein and a fragment of a 53BP2-IP protein, in which the fragment of the 53BP2 protein possesses the 53BP2-IP binding domain of the 53BP2 protein and the fragment of the 53BP2-IP protein possesses the 53BP2 binding domain of the 53BP2-IP protein, in which the 53BP2-IP is selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3.

8. A purified complex selected from the group consisting of: a complex of a derivative of a 53BP2 and a 53BP2-IP protein, a complex of a 53BP2 protein and a derivative of a 53BP2-IP, and a complex of a derivative of a 53BP2 and a derivative of a 53BP2-IP, in which said derivative of the 53BP2 protein is capable of forming a complex with a wild-type 53BP2-IP protein and said derivative of the 53BP2-IP is able to form a complex with a wild-type 53BP2 protein, in which the 53BP2-IP is selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3.

9. The purified complex of claim 8, wherein which said derivative of the 53BP2 protein and/or the 53BP2-IP protein is fluorescently-labeled.

10. A chimeric protein comprising a fragment of a 53BP2 protein consisting of at least 6 amino acid residues fused, via a covalent bond, to a fragment of a 53BP2-IP protein consisting of at least 6 amino acids.

11. The chimeric protein of claim 10, wherein said fragment of the 53BP2 protein is a fragment capable of binding the 53BP2-IP protein and wherein said fragment of the 53BP2-IP protein is a fragment capable of binding the 53BP2 protein.

12. The chimeric protein of claim 11, wherein said fragment of the 53BP2 protein and said fragment of the 53BP2-IP protein form a 53BP2•53BP2-IP complex.

13. An antibody which immunospecifically-binds a complex of a 53BP2 protein and a 53BP2-IP protein, or a fragment or derivative of said antibody possessing the binding domain thereof, in which the 53BP2-IP is selected from the group consisting of: β-tubulin, p62, hnRNP G. 53BP2:IP-1, 53BP2:IP-2 and 53BP2-IP-3.

14. The antibody of claim 13, which does not immunospecifically-bind a 53BP2 protein or a 53BP2-IP protein which are not part of a 53BP2•53BP2-IP complex.

15. An isolated nucleic acid or an isolated combination of nucleic acids comprising a nucleotide sequence encoding a 53BP2 protein and a nucleotide sequence encoding a 53BP2-IP protein selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3.

16. The isolated nucleic acid or isolated combination of nucleic acids of claim 15 which are nucleic acid vectors.

17. The isolated nucleic acid or isolated combination of nucleic acids of claim 16 wherein the 53BP2 protein-coding sequence and the 53BP2-IP protein-coding sequence are operably-linked to a promoter.

18. An isolated nucleic acid that comprises a nucleotide sequence encoding the chimeric protein of claim 12.

19. A recombinant cell containing said nucleic acid of claim 15.

20. A recombinant cell containing said nucleic acid of claim 17.

21. A recombinant cell containing said nucleic acid of claim 18.

22. A purified protein selected from the group consisting of: 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3.

23. The protein of claim 22 which is a human protein.

24. The protein of claim 23 which comprises an amino acid sequence selected from the group consisting of SEQ. ID NO:11. SEQ. ID NO:12 and SEQ. ID NO:13.

25. A purified protein encoded by a nucleic acid hybridizable to the inverse complement of a DNA possessing a nucleotide sequence consisting of SEQ. ID NO:10.

26. A purified derivative or analog of the protein of claim 22, wherein said derivative or analog can bind 53BP2.

27. The derivative or analog of claim 26 which is able to be bound by an antibody directed against a protein selected from the group consisting of: 53BP2:IP-l. 53BP2:IP-2 and 53BP2:IP-3.

28. A purified fragment of the protein of claim 22. wherein said fragment comprises a 53BP2 binding domain.

29. A purified protein comprising an amino acid sequence possessing at least 60% identity to the protein of claim 22, wherein the percentage identity is determined over an amino acid sequence of identical size to said protein of claim 22.

30. A chimeric protein comprising a fragment of the protein of claim 22, said fragment consisting of at least 6 amino acids, fused via a covalent bond to an amino acid sequence of a second protein, wherein the second protein is not said protein of claim 22.

31. An antibody which immunospecifically-binds the protein of claim 22, or a fragment or derivative of said antibody possessing the binding domain thereof.

32. An isolated nucleic acid comprising a nucleotide sequence encoding said protein of claim 22.

33. An isolated nucleic acid comprising the nucleotide sequence of SEQ. ID NO:10.

34. An isolated nucleic acid hybridizable to the inverse complement of the coding sequence of SEQ. ID NO:10.

35. A recombinant cell containing said nucleic acid of claim 34.

36. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of a complex of a 53BP2 protein and a 53BP2-IP protein, wherein said 53BP2-IP is selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3, and a pharmaceutically-acceptable carrier.

37. The pharmaceutical composition of claim 36 in which the proteins are human proteins.

38. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of a complex selected from the group consisting of: a complex of a fragment of a 53BP2 protein and a full-length 53BP2-IP protein. a complex of a full-length 53BP2 protein and a fragment of a 53BP2-IP protein, and a complex of a fragment of a 53BP2 protein and a fragment of a 53BP2-IP protein, wherein the fragment of said 53BP2 protein possesses the 53BP2-IP binding domain of the 53BP2 protein and the fragment of said 53BP2-IP protein possesses the 53BP2 binding domain of said 53BP2-IP protein, and wherein the 53BP2-IP is selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2-IP-3, and a pharmaceutically-acceptable carrier.

39. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of a chimeric protein comprising a fragment of a 53BP2 protein consisting of at least 6 amino acids fused via a covalent bond to a fragment of a 53BP2-IP protein consisting of at least 6 amino acids, and a pharmaceutically-acceptable carrier.

40. The pharmaceutical composition of claim 39 wherein the fragment of the 53BP2 protein is a fragment capable of binding the 53BP2-IP protein and wherein the fragment of the 53BP2-IP protein is capable of binding the 53BP2 protein.

41. The pharmaceutical composition of claim 40 wherein the fragment of the 53BP2 protein and the fragment of the 53BP2-IP protein form a 53BP2•53BP2-IP complex.

42. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of an antibody which immunospecifically-binds a complex of a 53BP2 protein and a 53BP2-IP protein. or a fragment or derivative of said antibody possessing the binding domain thereof. wherein the 53BP2-IP protein is selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2-IP-3, and a pharmaceutically-acceptable carrier.

43. The pharmaceutical composition of claim 42 wherein the antibody does not immunospecifically-bind a 53BP2 protein or a 53BP2-IP protein which are not part of a 53BP2•53BP2-IP complex.

44. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of a nucleic acid comprising a nucleotide sequence encoding a 53BP2 protein and a nucleotide sequence encoding a 53BP2-IP protein, wherein the 53BP2-IP is selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2-IP-3, and a pharmaceutically-acceptable carrier.

45. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of said isolated nucleic acid of claim 18, and a pharmaceutically-acceptable carrier.

46. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of said recombinant cell of claim 21, and a pharmaceutically-acceptable carrier.

47. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of a 53BP2:IP-l protein, 53BP2:IP-2 protein or 53BP2:IP-3 protein, and a pharmaceutically-acceptable carrier.

48. The pharmaceutical composition of claim 47, wherein the 53BP2:IP-l protein comprises the amino acid sequence as set forth in SEQ. ID NO:11; the 53BP2:IP-2 protein comprises the amino acid sequence as set forth in SEQ. ID NO:12 and the 53BP2:IP-3 protein comprises the amino acid sequence as set forth in SEQ. ID NO:13.

49. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of an antibody which immunospecifically-binds a protein selected from the group consisting of: a 53BP2:IP-l protein, a 53BP2:IP-2 protein and a 53BP2:IP-3 protein, or a fragment or derivative of said antibody possessing the binding domain thereof, and a pharmaceutically-acceptable carrier.

50. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of a nucleic acid comprising a nucleotide sequence encoding a protein selected from the group consisting of: 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, and a pharmaceutically-acceptable carrier.

51. A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of a recombinant cell containing said nucleic acid of claim 50, and a pharmaceutically-acceptable carrier.

52. A method of producing a complex of a 53BP2 protein and a 53BP2-IP protein comprising growing a recombinant cell containing the nucleic acid of claim 15, such that the encoded 53BP2 and 53BP2-IP proteins are expressed and bind to one another, and recovering the expressed complex of said 53BP2 protein and said 53BP2-IP protein.

53. A method of producing a protein selected from the group consisting of 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3 comprising growing a recombinant cell containing a nucleic acid

encoding said protein such that the encoded protein is expressed, and recovering the expressed protein.

54. A method of diagnosing or screening for the presence of or a predisposition for developing a disease or disorder characterized by an aberrant level of a complex of 53BP2 protein and a 53BP2-IP protein; wherein said 53BP2-IP is selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, in a subject comprising measuring the level of said complex, RNA encoding the 53BP2 and 53BP2-IP proteins, or functional activity of said complex in a sample derived from the subject. in which an increase or decrease in the level of said complex. said RNA encoding 53BP2 and 53BP2-IP, or functional activity of said complex in the sample. relative to the level of said complex. said RNA encoding 53BP2 and 53BP2-IP or functional activity of said complex found in an analogous sample not having the disease or disorder or a predisposition for developing the disease or disorder. indicates the presence of the disease or disorder or a predisposition for developing the disease or disorder.

55. A method of diagnosing or screening for the presence of or a predisposition for developing a disease or disorder characterized by an aberrant level of a protein or RNA selected from the group consisting of: 53BP2:IP-l. 53BP2:IP-2 or 53BP2:IP-3 protein or RNA in a subject. comprising measuring the level of said protein, said RNA or the functional activity of said protein in a sample derived from the subject; wherein an increase or decrease in the level of said protein, said RNA, or said functional activity in the sample, relative to the level of said protein. said RNA. or said functional activity found in an analogous sample not having the disease or disorder or a predisposition for developing the disease or. disorder. is indicative of the presence of the disease or disorder or a predisposition for developing the disease or disorder.

56. A kit comprising, in one or more containers, a substance selected from the group consisting of: a complex of 53BP2 and a 53BP2-IP, an antibody against said complex, nucleic acid probes capable of hybridizing to RNA of said 53BP2 and RNA of said 53BP2-IP, or pairs of nucleic acid primers capable of priming amplification of at least a portion of a gene for said 53BP2 and a gene for said 53BP2-IP, in which said 53BP2-IP is selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3.

57. A method of treating or preventing a disease or disorder involving aberrant levels of a complex of 53BP2 and 53BP2-IP. in which said 53BP2-IP is selected from the group consisting

of: β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2-IP-3, in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule or molecules which modulate the function of said complex.

58. The method of claim 57 in which said disease or disorder involves decreased levels of said complex and said molecule or molecules promote the function of the complex of 53BP2 and 53BP2-IP and are selected from the group consisting of a complex of 53BP2 and 53BP2-IP, a derivative or analog of a complex of 53BP2 and 53BP2-IP, which complex is more stable or more active than the wild-type complex, nucleic acids encoding the 53BP2 and 53BP2-IP proteins, and nucleic acids encoding a derivative or analog of 53BP2 and 53BP2-IP that form a complex that is more stable or more active than the wild-type complex.

59. The method of claim 57 in which said disease or disorder involves increased levels of said complex and said molecule or molecules inhibit the function of said complex and are selected from the group consisting of an antibody against said complex or a fragment or derivative thereof containing the binding region thereof, 53BP2 and 53BP2-IP antisense nucleic acids, and nucleic acids comprising at least a portion of a 53BP2 and a 53BP2-IP gene into which a heterologous nucleotide sequence has been inserted such that said heterologous sequence inactivates the biological activity of the at least a portion of the 53BP2 and 53BP2-IP genes, wherein the 53BP2 and the 53BP2-IP gene portions flank the heterologous sequences so as to promote homologous recombination with genomic 53BP2 and 53BP2-IP genes.

60. A method of treating or preventing a disease or disorder involving an aberrant level of a 53BP2-IP selected from the group consisting of: 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3 in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically-effective amount of a molecule which modulates the function of said 53BP2-IP.

61. The method of claim 60 in which said disease or disorder involves a decreased level of the 53BP2-IP and said molecule promotes the function of the 53BP2-IP and is selected from the group consisting of: the 53BP2-IP protein, a derivative or analog of the 53BP2-IP which is active in binding 53BP2, a nucleic acid encoding the 53BP2-IP protein, and a nucleic acid encoding a derivative or analog of the 53BP2-IP which is active in binding 53BP2.

62. The method of claim 60 in which said disease or disorder involves an increased level of the 53BP2-IP and said molecule inhibits the 53BP2-IP function and is selected from the group consisting of an anti-53BP2-IP antibody, or a fragment or derivative thereof possessing the binding region thereof, a 53BP2-IP antisense nucleic acid, and a nucleic acid comprising at least a portion of the 53BP2-IP gene into which a heterologous nucleotide sequence has been inserted such that said heterologous sequence inactivates the biological activity of the at least a portion of the 53BP2-IP gene, wherein the 53BP2-IP gene portion flanks the heterologous sequence so as to promote homologous recombination with a genomic 53BP2-IP gene.

63. A method of screening a purified complex of 53BP2 and a 53BP2-IP selected from the group consisting of: tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, or a derivative of said complex, or a modulator of the activity of said complex for anti-neoplastic activity comprising measuring the survival or proliferation of cells from a cell line which is derived from or displays characteristics associated with malignant disorder, which cells have been contacted with the complex, derivative, or modulator, and comparing the survival or proliferation in the cells contacted with the complex, derivative or modulator with said survival or proliferation in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator possesses anti-neoplastic activity.

64. A method of screening a purified complex of 53BP2 and a 53BP2-IP selected from the group consisting of: tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, or a derivative of said complex, or a modulator of the activity of said complex for anti-neoplastic activity by a method comprising administering the complex, derivative or modulator to a test animal, which test animal has a tumor, or which test animal does not have a tumor and is subsequently challenged with tumor cells or tumorigenic agents, and measuring tumor growth or regression in said test animal, wherein decreased tumor growth or increased tumor regression or prevention of tumor growth in test animals administered said complex, derivative or modulator compared to test animals not so administered indicates that the complex, derivative or modulator possesses anti-neoplastic activity.

65. A method for screening a purified complex of 53BP2 and a 53BP2-IP selected from the group consisting of; tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, or a derivative of said complex, or a modulator of the activity of said complex, for activity in treating

or preventing autoimmune disease comprising contacting cultured cells that exhibit an indicator of an autoimmune reaction *in vitro* with said complex, derivative or modulator, and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator possesses activity in treating or preventing autoimmune disease.

66. A method for screening a purified complex of 53BP2 and a 53BP2-IP selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing autoimmune disease comprising administering said complex, derivative or modulator to a test animal, which test animal exhibits an autoimmune reaction, or which test animal does not exhibit an autoimmune reaction and is subsequently challenged with an agent that elicits an autoimmune reaction, and measuring the change in the autoimmune reaction after the administration of said complex, derivative or modulator, wherein a reduction in said autoimmune reaction or prevention of said autoimmune reaction indicates that the complex, derivative or modulator possesses activity in treating or preventing an autoimmune disease.

67. A method for screening a purified complex of 53BP2 and a 53BP2-IP selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing neurodegenerative disease, comprising contacting cultured cells that exhibit an indicator of a neurodegenerative disease *in vitro* with said complex, derivative or modulator, and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator possesses activity in treating or preventing neurodegenerative disease.

68. A method for screening a purified complex of 53BP2 and a 53BP2-IP selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2—IP-3, or a derivative of said complex, or a modulator of the activity of said complex, for activity in treating or preventing neurodegenerative disease comprising administering said complex, derivative or modulator to a test animal, which test animal exhibits symptoms of a neurodegenerative disease

or which test animal is predisposed to develop symptoms of a neurodegenerative disease, and measuring the change in said symptoms of the neurodegenerative disease after administration of said complex, derivative, or modulator, wherein a reduction in the severity of the symptoms of the neurodegenerative disease or prevention of the symptoms of the neurodegenerative disease indicates that the complex, derivative or modulator possesses activity in treating or preventing neurodegenerative disease.

69. A method of screening for a molecule that modulates, directly or indirectly, the formation of a complex of 53BP2 and 53BP2-IP, in which said 53BP2-IP is selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3, comprising measuring the levels of said complex formed from 53BP2 and 53BP2-IP proteins in the presence of said molecule under conditions conducive to formation of the complex, and comparing the levels of said complex with the levels of said complex that are formed in the absence of said molecule, wherein a lower or higher level of said complex in the presence of said molecule indicates that the molecule modulates formation of said complex.

70. A recombinant, non-human animal in which both an endogenous 53BP2 gene and an endogenous 53BP2-IP gene selected from the group consisting of : β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3, have been deleted or inactivated by homologous recombination or insertional mutagenesis of said animal or an ancestor thereof.

71. A recombinant, non-human animal containing both a 53BP2 gene and a 53BP2-IP gene selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2-IP-3, wherein the 53BP2 gene is under the control of a promoter that is not the native 53BP2 gene promoter and the 53BP2-IP gene is under the control of a promoter that is not the native 53BP2-IP gene promoter.

72. A recombinant, non-human animal containing a transgene comprising a nucleic acid sequence encoding the chimeric protein of claim 11.

73. A recombinant, non-human animal in which an endogenous 53BP2-IP gene, selected from the group consisting of: a 53BP2:IP-1 gene, a 53BP2:IP-2 gene and a 53BP2:IP-3 gene, has

been deleted or inactivated by homologous recombination or insertional mutagenesis of said animal or an ancestor thereof.

74. A method of modulating the activity or levels of 53BP2 by contacting a cell with, or administering to an animal expressing a 53BP2 gene, a protein selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, or a nucleic acid encoding said protein or an antibody that immunospecifically-binds said protein or a fragment or derivative of said antibody possessing the binding domain thereof.

75. A method of modulating the activity or levels of a protein, selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, by contacting a cell with, or administering to an animal expressing a gene encoding said protein, 53BP2, or a nucleic acid encoding 53BP2, or an antibody that immunospecifically-binds 53BP2, or a fragment or derivative of said antibody possessing the binding domain thereof.

76. A method of modulating the activity or levels of a complex of 53BP2 and a protein selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, by contacting a cell with, or administering to an animal expressing and forming said complex, a molecule that modulates the formation of said complex.

77. A method for identifying a molecule that modulates activity of 53BP2, or a protein selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, or a complex of 53BP2 and said protein, which is comprised of contacting one or more candidate molecules with 53BP2 in the presence of said protein, and measuring the amount of complex which forms between 53BP2 and said protein; wherein an increase or decrease in the amount of complex which forms relative to the amount of complex which forms in the absence of the candidate molecules is indicative of the molecules possessing the ability to modulate the activity of 53BP2, or said protein, or said complex of 53BP2 and said protein.

78. The method of claim 77, wherein said contacting is carried out by administering the candidate molecules to the recombinant, non-human animal of claim 71.

79. The method of claim 77, wherein the candidate molecules are recombinantly-expressed in the recombinant, non-human animal of claim 71.

80. The method of claim 77, wherein said contacting is carried out *in vitro*, and wherein 53BP2, said protein, and said candidate molecules are substantially purified.

81. A method for screening a derivative or analog of 53BP2 for biological activity which is comprised of contacting said derivative or analog of 53BP2 with a protein selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3, and detecting the formation of a complex between said derivative or analog of 53BP2 and said protein; wherein detecting formation of said complex is indicative of said derivative or analog of 53BP2 possessing biological activity.

82. A method for screening a derivative or analog of a protein selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-1, 53BP2:IP-2 and 53BP2:IP-3, for biological activity comprising contacting said derivative or analog of said protein with 53BP2, and detecting the formation of a complex between said derivative or analog of said protein and 53BP2; wherein detecting the formation of said complex is indicative of said derivative or analog of said protein possessing biological activity.

83. A method of monitoring the efficacy of a treatment of a disease or disorder characterized by an aberrant level of a complex of the 53BP2 protein and a 53BP2-IP protein in a subject which has been administered said treatment for said disease or disorder, which is comprised of measuring the level of said complex, PITA encoding the 53BP2 and 53BP2-IP proteins, or functional activity of said complex within a sample derived from said subject; wherein said sample is taken from said subject after the administration of said treatment and compared to: (*i*) said level in a sample taken from said subject prior to the administration of the treatment or (*ii*) a standard level associated with the pre-treatment stage of the disease or disorder, in which the change, or lack of change in the level of said complex, said RNA encoding 53BP2 and 53BP2-IP, or functional activity of said complex within said sample taken after the administration of said treatment relative to the level of said complex, said RNA encoding 53BP2 and 53BP2-IP or functional activity of said complex in said sample taken before the

administration of said treatment or to said standard level is indicative of whether said administration is effective for treating said disease or disorder.

84. A method of treating or preventing cancer or a cell proliferation disorder in a subject which is comprised of administering to a subject in which such treatment or prevention is desired, a therapeutically-effective amount of a molecule that modulates the function of a complex of 53BP2 and a 53BP2-IP protein selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 or 53BP2:IP-3, or a combination of any one or more of the foregoing.

85. A method of treating or preventing neurodegenerative disease in a subject which is comprised of administering to a subject in which such treatment or prevention is desired, a therapeutically-effective amount of a molecule that modulates the function of a complex of 53BP2 and a 53BP2-IP protein selected from the group consisting of: β-tubulin, p62, hnRNP G, 53BP2:IP-l, 53BP2:IP-2 and 53BP2:IP-3, or a combination of any one or more of the foregoing.

86. A method of treating or preventing autoimmune disease in a subject which is comprised of administering to a subject in which such treatment or prevention is desired, a therapeutically-effective amount of a molecule that modulates the function of a complex of 53BP2 and hnRNP G.

87. A purified fragment of a protein selected from the group consisting of: β-tubulin, p62 and hnRNP G, wherein said fragment binds 53BP2.

```
GTCACGAGCG TCGAAGAGAC AAAGCCGCGT CAGGGGGCCC GGCCGGGGCG GGGGAGCCCG     60

GGGCTTGTTG GTGCCCCAGC CCGCGCGGAG GGCCCTTCGG ACCCGCGCGC CGCCGCTGCC    120

GCCGCCGCCG CCTCGCAACA GGTCCGGGCG GCCTCGCTCT CCGCTCCCCT CCCCCGCATC    180

CGCGACCCTC CGGGGCACCT CAGCTCGGCC GGGGCCGCAG TCTGGCCACC CGCTTCCATG    240

CGGTTCGGGT CCAAGATGAT GCCGATGTTT CTTACCGTGT ATCTCAGTAA CAATGAGCAG    300

CACTTCACAG AAGTTCCAGT TACTCCAGAA ACAATATGCA GAGACGTGGT GGATCTGTGC    360

AAAGAACCCG GCGAGAGTGA TTGCCATTTG GCTGAAGTGT GGTGTGGCTC TGTAGAGATA    420

GAGTTTCATC ATGTTGGCCA GGATGGTCTC GATCTCCTGA CCTTGTGATC CGCCTGCCTC    480

GGCCTCCCAA AGTGCTGGAT TACAGGTGTG AGCCACCACG ATCAGCCTCT AGTGTTTAAA    540

AAAGAACGTC CAGTTGCGGA TAATGAGCGA ATGTTTGATG TTCTTCAACG ATTTGGAAGT    600

CAGAGGAACG AAGTTCGCTT CTTCCTTCGT CATGAACGCC CCCCTGGCAG GGACATTGTG    660

AGTGGACCAA GATCTCAGGA TCCAAGTTTA AAAAGAAATG GTGTAAAAGT TCCTGGTGAA    720

TATCGAAGAA AGGAGAACGG TGTTAATAGT CCTAGG ATG GAT CTG ACT CTT GCT       774
                                        Met Asp Leu Thr Leu Ala
                                         1               5

GAA CTT CAG GAA ATG GCA TCT CGC CAG CAG CAA CAG ATT GAA GCC CAG       822
Glu Leu Gln Glu Met Ala Ser Arg Gln Gln Gln Gln Ile Glu Ala Gln
            10                  15                  20

CAA CAA TTG CTG GCA ACT AAG GAA CAG CGC TTA AAG TTT TTG AAA CAA       870
Gln Gln Leu Leu Ala Thr Lys Glu Gln Arg Leu Lys Phe Leu Lys Gln
        25                  30                  35

CAA GAT CAG CGA CAA CAG CAA CAA GTT GCT GAG CAG GAG AAA CTT AAA       918
Gln Asp Gln Arg Gln Gln Gln Gln Val Ala Glu Gln Glu Lys Leu Lys
    40                  45                  50

AGG CTA AAA GAA ATA GCT GAG AAT CAG GAA GCT AAG CTA AAA AAA GTG       966
Arg Leu Lys Glu Ile Ala Glu Asn Gln Glu Ala Lys Leu Lys Lys Val
55                  60                  65                  70

AGA GCA CTT AAA GGC CAC GTG GAA CAG AAG AGA CTA AGC AAT GGG AAA      1014
Arg Ala Leu Lys Gly His Val Glu Gln Lys Arg Leu Ser Asn Gly Lys
                75                  80                  85

CTT GTG GAG GAA ATT GAA CAG ATG AAT AAT TTG TTC CAG CAA AAA CAG      1062
Leu Val Glu Glu Ile Glu Gln Met Asn Asn Leu Phe Gln Gln Lys Gln
            90                  95                  100

AGG GAG CTC GTC CTG GCT GTG TCA AAA GTA GAA GAA CTG ACC AGG CAG      1110
Arg Glu Leu Val Leu Ala Val Ser Lys Val Glu Glu Leu Thr Arg Gln
        105                 110                 115
```

Fig. 1

```
CTA GAG ATG CTC AAG AAC GGC AGG ATC GAC AGC CAC CAT GAC AAT CAG    1158
Leu Glu Met Leu Lys Asn Gly Arg Ile Asp Ser His His Asp Asn Gln
    120                 125                 130

TCT GCA GTG GCT GAG CTT GAT CGC CTC TAT AAG GAG CTG CAG CTA AGA    1206
Ser Ala Val Ala Glu Leu Asp Arg Leu Tyr Lys Glu Leu Gln Leu Arg
135                 140                 145                 150

AAC AAA TTG AAT CAA GAG CAG AAT GCC AAG CTA CAA CAA CAG AGG GAG    1254
Asn Lys Leu Asn Gln Glu Gln Asn Ala Lys Leu Gln Gln Gln Arg Glu
                155                 160                 165

TGT TTG AAT AAG CGT AAT TCA GAA GTG GCA GTC ATG GAT AAG CGT GTT    1302
Cys Leu Asn Lys Arg Asn Ser Glu Val Ala Val Met Asp Lys Arg Val
            170                 175                 180

AAT GAG CTG AGG GAC CGG CTG TGG AAG AAG AAG GCA GCT CTA CAG CAA    1350
Asn Glu Leu Arg Asp Arg Leu Trp Lys Lys Lys Ala Ala Leu Gln Gln
        185                 190                 195

AAA GAA AAT CTA CCA GTT TCA TCT GAT GGA AAT CTT CCC CAG CAA GCC    1398
Lys Glu Asn Leu Pro Val Ser Ser Asp Gly Asn Leu Pro Gln Gln Ala
    200                 205                 210

GCG TCA GCC CCA AGC CGT GTG GCT GCA GTA GGT CCC TAT ATC CAG TCA    1446
Ala Ser Ala Pro Ser Arg Val Ala Ala Val Gly Pro Tyr Ile Gln Ser
215                 220                 225                 230

TCT ACT ATG CCT CGG ATG CCC TCA AGG CCT GAA TTG CTG GTG AAG CCA    1494
Ser Thr Met Pro Arg Met Pro Ser Arg Pro Glu Leu Leu Val Lys Pro
                235                 240                 245

GCC CTG CCG GAT GGT TCC TTG GTC ATT CAG GCT TCA GAG GGG CCG ATG    1542
Ala Leu Pro Asp Gly Ser Leu Val Ile Gln Ala Ser Glu Gly Pro Met
            250                 255                 260

AAA ATA CAG ACA CTG CCC AAC ATG AGA TCT GGG GCT GCT TCA CAA ACT    1590
Lys Ile Gln Thr Leu Pro Asn Met Arg Ser Gly Ala Ala Ser Gln Thr
        265                 270                 275

AAA GGC TCT AAA ATC CAT CCA GTT GGC CCT GAT TGG AGT CCT TCA AAT    1638
Lys Gly Ser Lys Ile His Pro Val Gly Pro Asp Trp Ser Pro Ser Asn
    280                 285                 290

GCA GAT CTT TTC CCA AGC CAA GGC TCT GCT TCT GTA CCT CAA AGC ACT    1686
Ala Asp Leu Phe Pro Ser Gln Gly Ser Ala Ser Val Pro Gln Ser Thr
295                 300                 305                 310

GGG AAT GCT CTG GAT CAA GTT GAT GAT GGA GAG GTT CCG CTG AGG GAG    1734
Gly Asn Ala Leu Asp Gln Val Asp Asp Gly Glu VAl Pro Leu Arg Glu
                315                 320                 325
```

Fig. 1 (cont.)

```
AAA GAG AAG AAA GTG CGT CCG TTC TCA ATG TTT GAT GCA GTA GAC CAG    1782
Lys Glu Lys Lys Val Arg Pro Phe Ser Met Phe Asp Ala Val Asp Gln
            330                 335                 340

TCC AAT GCC CCA CCT TCC TTT GGT ACT CTG AGG AAG AAC CAG AGC AGT    1830
Ser Asn Ala Pro Pro Ser Phe Gly Thr Leu Arg Lys Asn Gln Ser Ser
        345                 350                 355

GAA GAT ATC TTG CGG GAT GCT CAG GTT GCA AAT AAA AAT GTG GCT AAA    1878
Glu Asp Ile Leu Arg Asp Ala Gln Val Ala Asn Lys Asn Val Ala Lys
    360                 365                 370

GTA CCA CCT CCT GTT CCT ACA AAA CCA AAA CAG ATT AAT TTG CCT TAT    1926
Val Pro Pro Pro Val Pro Thr Lys Pro Lys Gln Ile Asn Leu Pro Tyr
375                 380                 385                 390

TTT GGA CAA ACT AAT CAG CCA CCT TCA GAC ATT AAG CCA GAC GGA AGT    1974
Phe Gly Gln Thr Asn Gln Pro Pro Ser Asp Ile Lys Pro Asp Gly Ser
                395                 400                 405

TCT CAG CAG TTG TCA ACA GTT GTT CCG TCC ATG GGA ACT AAA CCA AAA    2022
Ser Gln Gln Leu Ser Thr Val Val Pro Ser Met Gly Thr Lys Pro Lys
            410                 415                 420

CCA GCA GGG CAG CAG CCG AGA GTG CTG CTA TCT CCC AGC ATA CCT TCG    2070
Pro Ala Gly Gln Gln Pro Arg Val Leu Leu Ser Pro Ser Ile Pro Ser
        425                 430                 435

GTT GGC CAA GAC CAG ACC CTT TCT CCA GGT TCT AAG CAA GAA AGT CCA    2118
Val Gly Gln Asp Gln Thr Leu Ser Pro Gly Ser Lys Gln Glu Ser Pro
    440                 445                 450

CCT GCT GCT GCC GTC CGG CCC TTT ACT CCC CAG CCT TCC AAA GAC ACC    2166
Pro Ala Ala Ala Val Arg Pro Phe Thr Pro Gln Pro Ser Lys Asp Thr
455                 460                 465                 470

TTA CTT CCA CCC TTC AGA AAA CCC CAG ACC GTG GCA GCA AGT TCA ATA    2214
Leu Leu Pro Pro Phe Arg Lys Pro Gln Thr Val Ala Ala Ser Ser Ile
                475                 480                 485

TAT TCC ATG TAT ACG CAA CAG CAG GCG CCA GGA AAA AAC TTC CAG CAG    2262
Tyr Ser Met Tyr Thr Gln Gln Gln Ala Pro Gly Lys Asn Phe Gln Gln
            490                 495                 500

GCT GTG CAG AGC GCG TTG ACC AAG ACT CAT ACC AGA GGG CCA CAC TTT    2310
Ala Val Gln Ser Ala Leu Thr Lys Thr His Thr Arg Gly Pro His Phe
        505                 510                 515

TCA AGT GTA TAT GGT AAG CCT GTA ATT GCT GCT GCC CAG AAT CAA CAG    2358
Ser Ser Val Tyr Gly Lys Pro Val Ile Ala Ala Ala Gln Asn Gln Gln
    520                 525                 530
```

Fig. 1 (cont.)

```
CAG CAC CCA GAG AAC ATT TAT TCC AAT AGC CAG GGC AAG CCT GGC AGT    2406
Gln His Pro Glu Asn Ile Tyr Ser Asn Ser Gln Gly Lys Pro Gly Ser
535                 540                 545                 550

CCA GAA CCT GAA ACA GAG CCT GTT TCT TCA GTT CAG GAG AAC CAT GAA    2454
Pro Glu Pro Glu Thr Glu Pro Val Ser Ser Val Gln Glu Asn His Glu
                555                 560                 565

AAC GAA AGA ATT CCT CGG CCA CTC AGC CCA ACT AAA TTA CTG CCT TTC    2502
Asn Glu Arg Ile Pro Arg Pro Leu Ser Pro Thr Lys Leu Leu Pro Phe
            570                 575                 580

TTA TCT AAT CCT TAC CGA AAC CAG AGT GAT GCT GAC CTA GAA GCC TTA    2550
Leu Ser Asn Pro Tyr Arg Asn Gln Ser Asp Ala Asp Leu Glu Ala Leu
        585                 590                 595

CGA AAG AAA CTG TCT AAC GCA CCA AGG CCT CTA AAG AAA CGT AGT TCT    2598
Arg Lys Lys Leu Ser Asn Ala Pro Arg Pro Leu Lys Lys Arg Ser Ser
    600                 605                 610

ATT ACA GAG CCA GAG GGT CCT AAT GGG CCA AAT ATT CAG AAG CTT TTA    2646
Ile Thr Glu Pro Glu Gly Pro Asn Gly Pro Asn Ile Gln Lys Leu Leu
615                 620                 625                 630

TAT CAG AGG ACC ACC ATA GCG GCC ATG GAG ACC ATC TCT GTC CCA TCA    2694
Tyr Gln Arg Thr Thr Ile Ala Ala Met Glu Thr Ile Ser Val Pro Ser
                635                 640                 645

TAC CCA TCC AAG TCA GCT TCT GTG ACT GCC AGC TCA GAA AGC CCA GTA    2742
Tyr Pro Ser Lys Ser Ala Ser Val Thr Ala Ser Ser Glu Ser Pro Val
            650                 655                 660

GAA ATC CAG AAT CCA TAT TTA CAT GTG GAG CCC GAA AAG GAG GTG GTC    2790
Glu Ile Gln Asn Pro Tyr Leu His Val Glu Pro Glu Lys Glu Val Val
        665                 670                 675

TCT CTG GTT CCT GAA TCA TTG TCC CCA GAG GAT GTG GGG AAT GCC AGT    2838
Ser Leu Val Pro Glu Ser Leu Ser Pro Glu Asp Val Gly Asn Ala Ser
    680                 685                 690
                                A
ACA GAG AAC AGT GAC ATG CCA GCT CCT TCT CCA GGC CTT GAT TAT GAG    2886
Thr Glu Asn Ser Asp Met Pro Ala Pro Ser Pro Gly Leu Asp Tyr Glu
695                 700                 705                 710

CCT GAG GGA GTC CCA GAC AAC AGC CCA AAT CTC CAG AAT AAC CCA GAA    2934
Pro Glu Gly Val Pro Asp Asn Ser Pro Asn Leu Gln Asn Asn Pro Glu
                715                 720                 725

GAA CCA AAT CCA GAG GCT CCA CAT GTG CTT GAT GTG TAC CTG GAG GAG    2982
Glu Pro Asn Pro Glu Ala Pro His Val Leu Asp Val Tyr Leu Glu Glu
            730                 735                 740
```

Fig. 1 (cont.)

```
TAC CCT CCA TAC CCA CCC CCA CCA TAC CCA TCT GGG GAG CCT GAA GGG    3030
Tyr Pro Pro Tyr Pro Pro Pro Pro Tyr Pro Ser Gly Glu Pro Glu Gly
        745                 750                 755

CCC GGA GAA GAC TCG GTG AGC ATG CGC CCG CCT GAA ATC ACC GGG CAG    3078
Pro Gly Glu Asp Ser Val Ser Met Arg Pro Pro Glu Ile Thr Gly Gln
    760                 765                 770

GTC TCT CTG CCT CCT GGT AAA AGG ACA AAC TTG CGT AAA ACT GGC TCA    3126
Val Ser Leu Pro Pro Gly Lys Arg Thr Asn Leu Arg Lys Thr Gly Ser
775                 780                 785                 790

GAG CGT ATC GCT CAT GGA ATG AGG GTG AAA TTC AAC CCC CTT GCT TTA    3174
Glu Arg Ile Ala His Gly Met Arg Val Lys Phe Asn Pro Leu Ala Leu
                795                 800                 805

CTG CTA GAT TCG TCT TTG GAG GGA GAA TTT GAC CTT GTA CAG AGA ATT    3222
Leu Leu Asp Ser Ser Leu Glu Gly Glu Phe Asp Leu Val Gln Arg Ile
            810                 815                 820

ATT TAT GAG GTT GAT GAC CCA AGC CTG CCC AAT GAT GAA GGC ATC ACG    3270
Ile Tyr Glu Val Asp Asp Pro Ser Leu Pro Asn Asp Glu Gly Ile Thr
        825                 830                 835

GCT CTT CAC AAT GCT GTG TGT GCA GGC CAC ACA GAA ATC GTT AAG TTC    3318
Ala Leu His Asn Ala Val Cys Ala Gly His Thr Glu Ile Val Lys Phe
    840                 845                 850

CTG GTA CAG TTT GGT GTA AAT GTA AAT GCT GCT GAT AGT GAT GGA TGG    3366
Leu Val Gln Phe Gly Val Asn Val Asn Ala Ala Asp Ser Asp Gly Trp
855                 860                 865                 870

ACT CCA TTA CAT TGT GCT GCC TCA TGT AAC AAC GTC CAA GTG TGT AAG    3414
Thr Pro Leu His Cys Ala Ala Ser Cys Asn Asn Val Gln Val Cys Lys
                875                 880                 885

TTT TTG GTG GAG TCA GGA GCC GCT GTG TTT GCC ATG ACC TAC AGT GAC    3462
Phe Leu Val Glu Ser Gly Ala Ala Val Phe Ala Met Thr Tyr Ser Asp
            890                 895                 900

ATG CAG ACT GCT GCA GAT AAG TGC GAG GAA ATG GAG GAA GGC TAC ACT    3510
Met Gln Thr Ala Ala Asp Lys Cys Glu Glu Met Glu Glu Gly Tyr Thr
        905                 910                 915

CAG TGC TCC CAA TTT CTT TAT GGA GTT CAG GAG AAG ATG GGC ATA ATG    3558
Gln Cys Ser Gln Phe Leu Tyr Gly Val Gln Glu Lys Met Gly Ile Met
    920                 925                 930

AAT AAA GGA GTC ATT TAT GCG CTT TGG GAT TAT GAA CCT CAG AAT GAT    3606
Asn Lys Gly Val Ile Tyr Ala Leu Trp Asp Tyr Glu Pro Gln Asn Asp
935                 940                 945                 950
```

Fig. 1 (cont.)

```
GAT GAG CTG CCC ATG AAA GAA GGA GAC TGC ATG ACA ATC ATC CAC AGG    3654
Asp Glu Leu Pro Met Lys Glu Gly Asp Cys Met Thr Ile Ile His Arg
               955                 960                 965

GAA GAC GAA GAT GAA ATC GAA TGG TGG TGG GCG CGC CTT AAT GAT AAG    3702
Glu Asp Glu Asp Glu Ile Glu Trp Trp Trp Ala Arg Leu Asn Asp Lys
           970                 975                 980

GAG GGA TAT GTT CCA CGT AAC TTG CTG GGA CTG TAC CCA AGA ATT AAA    3750
Glu Gly Tyr VAl Pro Arg Asn Leu Leu Gly Leu Tyr Pro Arg Ile Lys
       985                 990                 995

CCA AGA CAA AGG AGC TTG GCC TGAAACTTCC ACACAGAATT TTAGTCAATG AAGA  3805
Pro Arg Gln Arg Ser Leu Ala
   1000                1005

ATTAATCTCT GTTAAGAAGA AGTAATACGA TTATTTTTGG CAAAAATTTC ACAAGACTTA  3865

TTTTAATGAC AATGTAGCTT GAAAGCGATG AAGAATGTCT CTAGAAGAGA ATGAAGGATT  3925

GAAGAATTCA CCATTAGAGG ACATTTAGCG TGATGAAATA AAGCATCTAC GTCAGCAGGC  3985

CATACTGTGT TGGGGCAAAG GTGTCCCGTG TAGCACTCAG ATAAGTATAC AGCGACAATC  4045

CTGTTTTCTA CAAGAATCCT GTCTAGTAAA TAGGATCATT TATTGGGCAG TTGGGAAATG  4105

AGCTCTCTGT CCTGTTGAGT GTTTTCAGCA GCTGCTCCTA AACCAGTCCT CCTGCCAGAA  4165

AGGACCAGTG CCGTCACATC GCTGTCTCTG ATTGTCCCCG GCACCAGCAG GCCTTGGGGC  4225

TCACTGAAGG CTCGAAGGCA CTGCACACCT TGTATATTGT CAGTGAAGAA CGTTAGTTGG  4285

TTGTCAGTGA ACAATAACTT TATTATATGA GTTTTTGTAG CATCTTAAGA ATTATACATA  4345

TGTTTGAAAT ATTGAAACTA AGCTACAGTA CCAGTAATTA GATGTAGAAT CTTGTTTGTA  4405

GGCTGAATTT TAATCTGTAT TTATTGTCTT TTGTATCTCA GAAATTAGAA ACTTGCTACA  4465

GACTTACCCG TAATATTTGT CAAGATCATA GCTGACTTTA AAAACAGTTG TAATAAACTT  4525

TTTGATGCT                                                          4534
```

Fig. 1 (cont.)

```
GCCCGCCGGT CCACGCCGCG CACCGCTCCG AGGGCCAGCG CCACCCGCTC CGCAGCCGGC    60

ACC ATG CGC GAG ATC GTG CAC ATC CAG GCG GGC CAG TGC GGC AAC CAG    108
    Met Arg Glu Ile Val His Ile Gln Ala Gly Gln Cys Gly Asn Gln
     1               5                  10                  15

ATC GGC GCC AAG TTT TGG GAG GTC ATC AGC GAT GAG CAT GGG ATC GAC    156
Ile Gly Ala Lys Phe Trp Glu Val Ile Ser Asp Glu His Gly Ile Asp
                20                  25                  30

CCC ACA GGC AGT TAC CAT GGA GAC AGT GAC TTG CAG CTG GAG AGA ATC    204
Pro Thr Gly Ser Tyr His Gly Asp Ser Asp Leu Gln Leu Glu Arg Ile
            35                  40                  45

AAC GTG TAC TAC AAT GAG GCT GCT GGT AAC AAA TAT GTA CCT CGG GCC    252
Asn Val Tyr Tyr Asn Glu Ala Ala Gly Asn Lys Tyr Val Pro Arg Ala
        50                  55                  60

ATC CTG GTG GAT CTG GAG CCT GGC ACC ATG GAC TCT GTC AGG TCT GGA    300
Ile Leu Val Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg Ser Gly
    65                  70                  75

CCC TTC GGC CAG ATC TTC AGA CCA GAC AAC TTC GTG TTC GGC CAG AGT    348
Pro Phe Gly Gln Ile Phe Arg Pro Asp Asn Phe Val Phe Gly Gln Ser
80                  85                  90                  95

GGA GCC GGG AAT AAC TGG GCC AAG GGC CAC TAC ACA GAG GGA GCC GAG    396
Gly Ala Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly Ala Glu
                100                 105                 110

CTG GTC GAC TCG GTC CTG GAT GTG GTG AGG AAG GAG TCA GAG AGC TGT    444
Leu Val Asp Ser Val Leu Asp Val Val Arg Lys Glu Ser Glu Ser Cys
            115                 120                 125

GAC TGT CTC CAG GGC TTC CAG CTG ACC CAC TCT CTG GGG GGC GGC ACG    492
Asp Cys Leu Gln Gly Phe Gln Leu Thr His Ser Leu Gly Gly Gly Thr
        130                 135                 140

GGG TCC GGG ATG GGC ACC CTG CTC ATC AGC AAG ATC CGG GAA GAG TAC    540
Gly Ser Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg Glu Glu Tyr
    145                 150                 155

CCA GAC CGC ATC ATG AAC ACC TTC AGC GTC ATG CCC TCA CCC AAG GTG    588
Pro Asp Arg Ile Met Asn Thr Phe Ser Val Met Pro Ser Pro Lys Val
160                 165                 170                 175

TCA GAC ACG GTG GTG GAG CCC TAC AAC GCC ACC CTC TCG GTC CAC CAG    636
Ser Asp Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser Val His Gln
                180                 185                 190

CTG GTG GAA AAC ACA GAT GAA ACC TAC TCC ATT GAT AAC GAG GCC CTG    684
Leu Val Glu Asn Thr Asp Glu Thr Tyr Ser Ile Asp Asn Glu Ala Leu
            195                 200                 205
```

Fig. 2

```
TAT GAC ATC TGC TTC CGC ACC CTG AAG CTG ACC ACC CCC ACC TAC GGG      732
Tyr Asp Ile Cys Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly
        210                 215                 220

GAC CTC AAC CAC CTG GTG TCG GCC ACC ATG AGC GGG GTC ACC ACC TGC      780
Asp Leu Asn His Leu Val Ser Ala Thr Met Ser Gly Val Thr Thr Cys
    225                 230                 235          (A)

CTG CGC TTC CCG GGC CAG CTG AAC GCA GAC CTG CGC AAG CTG GCG GTG      828
Leu Arg Phe Pro Gly Gln Leu Asn Ala Asp Leu Arg Lys Leu Ala Val
240                 245                 250                 255

AAC ATG GTG CCC TTC CCT CGC CTG CAC TTC TTC ATG CCC GGC TTC GCG      876
Asn Met Val Pro Phe Pro Arg Leu His Phe Phe Met Pro Gly Phe Ala
                260          (B)    265                 270

CCC CTG ACC AGC CGG GGC AGC CAG CAG TAC CGG GCG CTC ACG GTG CCC      924
Pro Leu Thr Ser Arg Gly Ser Gln Gln Tyr Arg Ala Leu Thr Val Pro
            275                 280                 285

GAG CTC ACC CAG CAG ATG TTC GAC TCC AAG AAC ATG ATG GCC GCC TGC      972
Glu Leu Thr Gln Gln Met Phe Asp Ser Lys Asn Met Met Ala Ala Cys
        290                 295                 300

GAC CCG CGC CAC GGC CGC TAC CTG ACG GTG GCT GCC ATC TTC CGG GGC     1020
Asp Pro Arg His Gly Arg Tyr Leu Thr Val Ala Ala Ile Phe Arg Gly
    305                 310                 315

CGC ATG TCC ATG AAG GAG GTG GAC GAG CAG ATG CTC AAC GTG CAG AAC     1068
Arg Met Ser Met Lys Glu Val Asp Glu Gln Met Leu Asn Val Gln Asn
320                 325                 330                 335

AAG AAC AGC AGC TAC TTC GTG GAG TGG ATC CCC AAC AAC GTG AAG ACG     1116
Lys Asn Ser Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn Val Lys Thr
                340                 345                 350

GCC GTG TGC GAC ATC CCG CCC CGC GGC CTG AAG ATG TCG GCC ACC TTC     1164
Ala Val Cys Asp Ile Pro Pro Arg Gly Leu Lys Met Ser Ala Thr Phe
            355                 360                 365

ATC GGC AAC AGC ACG GCC ATC CAG GAG CTG TTC AAG CGC ATC TCC GAG     1212
Ile Gly Asn Ser Thr Ala Ile Gln Glu Leu Phe Lys Arg Ile Ser Glu
        370                 375                 380

CAG TTC ACG GCC ATG TTC CGG CGC AAG GCC TTC CTG CAC TGG TAC ACG     1260
Gln Phe Thr Ala Met Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr
    385                 390                 395

GGC GAG GGC ATG GAC GAG ATG GAG TTC ACC GAG GCC GAG AGC AAC ATG     1308
Gly Glu Gly Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met
400                 405                 410                 415
```

Fig. 2 (cont.)

```
AAC GAC CTG GTG TCC GAG TAC CAG CAG TAC CAG GAC GCC ACG GCC GAC    1356
Asn Asp Leu Val Ser Glu Tyr Gln Gln Tyr Gln Asp Ala Thr Ala Asp
                420                 425                 430

GAA CAA GGG GAG TTC GAG GAG GAG GAG GGC GAG GAC GAG G CTTAAAAACT   1406
Glu Gln Gly Glu Phe Glu Glu Glu Glu Gly Glu Asp Glu Ala
            435                 440                 445

TCTCAGATCA ATCGTGCATC CTTAGTGAAC TTCTGTTGTC CTCAAGCATG GTCTTTCTAC   1466

TTGTAAACTA TGGTGCTCAG TTTTGCCTCT GTTAGAAATT CACACTGTTG ATGTAATGAT   1526

GTGGAACTCC TCTAAAAATT ACAGTATTGT CTGTGAAGGT ATCTATACTA ATAAAAAAGC   1586

ATGTGTAG                                                            1594
```

Fig. 2 (cont.)

```
GGCTTCGGTC GCTACCGCTC CCGCTCTGCC ACCCCCGCCA ACCGCCGCTC GGGCCTCCGT    60

CGCTGCCGCG TCGCTTTCTC GCTCCTTGGA TCGCACATCC TCCCAG ATG CAG CGC       115
                                                   Met Gln Arg
                                                   1

CGG GAC GAC CCC GCC GCG CGC ATG AGC CGG TCT TCG GGC CGT AGC GGC       163
Arg Asp Asp Pro Ala Ala Arg Met Ser Arg Ser Ser Gly Arg Ser Gly
    5                  10                  15

TCC ATG GAC CCC TCC GGT GCC CAC CCC TCG GTG CGT CAG ACG CCG TCT       211
Ser Met Asp Pro Ser Gly Ala His Pro Ser Val Arg Gln Thr Pro Ser
20                  25                  30                  35

CGG CAG CCG CCG CTG CCT CAC CGG TCC CGG GGA GGC GGA GGG GGA TCC       259
Arg Gln Pro Pro Leu Pro His Arg Ser Arg Gly Gly Gly Gly Gly Ser
40                  45                  50

CGC GGG GGC CGG CGG GCC TCG CCC GCC ACG CAG CCG CCA CCG CTG CTG       307
Arg Gly Gly Ala Arg Ala Ser Pro Ala Thr Gln Pro Pro Pro Leu Leu
55                  60                  65

CCG CCC TCG GCC ACG GGT CCC GAC GCG ACA GTG GGC GGG CCA GCG CCG       355
Pro Pro Ser Ala Thr Gly Pro Asp Ala Thr Val Gly Gly Pro Ala Pro
70                  75                  80

ACC CCG CTG CTG CCC CCC TCG GCC ACA GCC TCG GTC AAG ATG GAG CCA       403
Thr Pro Leu Leu Pro Pro Ser Ala Thr Ala Ser Val Lys Met Glu Pro
85                  90                  95

GAG AAC AAG TAC CTG CCC GAA CTC ATG GCC GAG AAG GAC TCG CTC GAC       451
Glu Asn Lys Tyr Leu Pro Glu Leu Met Ala Glu Lys Asp Ser Leu Asp
100                 105                 110                 115

CCG TCC TTC ACT CAC GCC ATG CAG CTG CTG ACG GCA GAA ATT GAG AAG       499
Pro Ser Phe Thr His Ala Met Gln Leu Leu Thr Ala Glu Ile Glu Lys
120                 125                 130

ATT CAG AAA GGA GAC TCA AAA AAG GAT GAT GAG GAG AAT TAC TTG GAT       547
Ile Gln Lys Gly Asp Ser Lys Lys Asp Asp Glu Glu Asn Tyr Leu Asp
135                 140                 145

TTA TTT TCT CAT AAG AAC ATG AAA CTG AAA GAG CGA GTG CTG ATA CCT       595
Leu Phe Ser His Lys Asn Met Lys Leu Lys Glu Arg Val Leu Ile Pro
150                 155                 160

GTC AAG CAG TAT CCC AAG TTC AAT TTT GTG GGG AAG ATT CTT GGA CCA       643
Val Lys Gln Tyr Pro Lys Phe Asn Phe Val Gly Lys Ile Leu Gly Pro
165                 170                 175
```

Fig. 3

```
CAA GGG AAT ACA ATC AAA AGA CTG CAG GAA GAG ACT GGT GCA AAG ATC      691
Gln Gly Asn Thr Ile Lys Arg Leu Gln Glu Glu Thr Gly Ala Lys Ile
180                 185                 190                 195

TCT GTA TTG GGA AAG GGC TCA ATG AGA GAC AAA GCC AAG GAG GAA GAG      739
Ser Val Leu Gly Lys Gly Ser Met Arg Asp Lys Ala Lys Glu Glu Glu
200                 205                 210

CTG CGC AAA GGT GGA GAC CCC AAA TAT GCC CAC TTG AAT ATG GAT CTG      787
Leu Arg Lys Gly Gly Asp Pro Lys Tyr Ala His Leu Asn Met Asp Leu
215                 220                 225

CAT GTC TTC ATT GAA GTC TTT GGA CCC CCA TGT GAG GCT TAT GCT CTT      835
His Val Phe Ile Glu Val Phe Gly Pro Pro Cys Glu Ala Tyr Ala Leu
230                 235                 240

ATG GCC CAT GCC ATG GAG GAA GTC AAG AAA TTT CTA GTA CCG GAT ATG      883
Met Ala His Ala Met Glu Glu Val Lys Lys Phe Leu Val Pro Asp Met
245                 250                 255                 (A)

ATG GAT GAT ATC TGT CAG GAG CAA TTT CTA GAG CTG TCC TAC TTG AAT      931
Met Asp Asp Ile Cys Gln Glu Gln Phe Leu Glu Leu Ser Tyr Leu Asn

260                 265                 270                 275
GGA GTA CCT GAA CCC TCT CGT GGA CGT GGG GTG CCA GTG AGA GGC CGG      979
Gly Val Pro Glu Pro Ser Arg Gly Arg Gly Val Pro Val Arg Gly Arg
280                 285                 290

GGA GCT GCA CCT CCT CCA CCA CCT GTT CCC AGG GGC CGT GGT GTT GGA     1027
Gly Ala Ala Pro Pro Pro Pro Pro Val Pro Arg Gly Arg Gly Val Gly
295                 300                 305

CCA CCT CGG GGG GCT TTG GTA CGT GGT ACA CCA GTA AGG GGA GCC ATC     1075
Pro Pro Arg Gly Ala Leu Val Arg Gly Thr Pro Val Arg Gly Ala Ile
310                 315                 320

ACC AGA GGT GCC ACT GTG ACT CGA GGC GTG CCA CCC CCA CCT ACT GTG     1123
Thr Arg Gly Ala Thr Val Thr Arg Gly Val Pro Pro Pro Pro Thr Val
325                 330                 335

AGG GGT GCT CCA GCA CCA AGA GCA CGG ACA GCG GGC ATC CAG AGG ATA     1171
Arg Gly Ala Pro Ala Pro Arg Ala Arg Thr Ala Gly Ile Gln Arg Ile
340                 345                 350                 355

CCT TTG CCT CCA CCT CCT GCA CCA GAA ACA TAT GAA GAA TAT GGA TAT     1219
Pro Leu Pro Pro Pro Pro Ala Pro Glu Thr Tyr Glu Glu Tyr Gly Tyr
360                 365                 370

GAT GAT ACA TAC GCA GAA CAA AGT TAC GAA GGC TAC GAA GGC TAT TAC     1267
Asp Asp Thr Tyr Ala Glu Gln Ser Tyr Glu Gly Tyr Glu Gly Tyr Tyr
375                 380                 385

AGC CAG AGT CAA GGG GAC TCA GAA TAT TAT GAC TAT GGA CAT GGG GAG     1315
Ser Gln Ser Gln Gly Asp Ser Glu Tyr Tyr Asp Tyr Gly His Gly Glu
390                 395                 400
```

Fig. 3 (cont.)

```
GTT CAA GAT TCT TAT GAA GCT TAT GGC CAG GAC GAC TGG AAT GGG ACC    1363
Val Gln Asp Ser Tyr Glu Ala Tyr Gly Gln Asp Asp Trp Asn Gly Thr
405                 410                 415

AGG CCG TCG CTG AAG GCC CCT CCT GCT AGG CCA GTG AAG GGA GCA TAC    1411
Arg Pro Ser Leu Lys Ala Pro Pro Ala Arg Pro Val Lys Gly Ala Tyr
420                 425                 430                 435

AGA GAG CAC CCA TAT GGA CGT TAT TAAAAACAAA CATGAGGGGA AAATATCAGT   1465
Arg Glu His Pro Tyr Gly Arg Tyr
                440         443

TATGAGCAAA GTTGTTACTG ATTTCTTGTA TCTCCCAGGA TTCCTGTTGC TTTACCCACA  1525

ACAGACAAGT AATTGTCTAA GTGTTTTTCT TCGTGGTCCC CTTCTTCTCC CCACCTTATT  1585

CCATTCTTAA CTCTGCATTC TGGCTTCTGT ATGTAGTATT TTAAAATGAG TTAAAATAGA  1645

TTTAGGAATA TTGAATTAAT TTTTTAAGTG TGTAGATGCT TTTTTCTTTG TTGTTTAAAT  1705

ATAAACAGAA GTGTACCTTT TATAATAAAA AAAAGAAGTT GAGTAAAAAA AAAAAACACA  1765

CAAACCTGTT AGTTTCAAAA ATGACATTGC TTGCTTAAAG GTTCTGAAGT AAAGGCTTGT  1825

TAAGTTTCTC TTAGTTTTGA TTTGAGGCAT CCCGTAAAGT TGTAGTTGCA GAATCCCAAA  1885

CTAGGCTACA TTTCAAAATT CAGGGCTGTT TAAGATTTAA AATCACAAAC ATTAACGGCA  1945

GTAGGCACCA CCATGTAAAA GTGAGCTCAG ACGTCTCTAA AAAATGTTTC CTTTATAAAA  2005

GCACATGGCG GTTGAATCTT AAGGTTAAAT TTTAATATGA AAGATCCTCA TGAATTAAAT  2065

AGTTGATGCA ATTTTTAACG TTAATTGATA TAAAAAAAAA AACAACAAAA TTAGGCTTGT  2125

AAAACTGACT TTTTCATTAC GTGGGTTTTG AAATCTAGCC CCAGACATAC TGTGTTGAGA  2185

GATACTTAGA GGGAGGGAGT AGGTTTTGAA GAGGTTGATG GTGGTGGGGA GGGAAGGCCT  2245

CCTGAATTGA GTTTGATGCA GAGCTTTTTA GCCATGAAGA ATCTTTCAGT CATAGTACTA  2305

ATAATTAAAT TTTCAGTATT TAAAAAGACA AAGTATTTTG TCCATTTGAG ATTCTGCACT  2365

CCATGAAAAG TTCACTTGGA CGCTGGGGCC AAAAGCTGTT GATTTTCTTA AGTTGACGGT  2425

TGTCAATATA TCGAACTGTT CCCAAGTTAG TCAAGTATGT CTCAACACTA GCATGATATA  2485

AAAAGGGACA CTGCAGCTGA ATGAAAAAGG AATCAAAATC CACTTTGTAC ATAAGTTAAA  2545

GTCCTAATTG GATTTGTACC GTCCTCCCAT TTTGTTCTCG GAAGATTAAA TGCTACATGT  2605

GTAAGTCTGC CTAAATAGGT AGCTTAAACT TATGTCAAAA TGTCTGCAGC AGTTTGTCAA  2665

TAAAGTTTAG TCCTTTTTTA                                              2685
```

Fig. 3 (cont.)

```
CGGAAAAAAA A ATG GTT GAA GCA GAT CGC CCA GGA AAG CTC TTC ATT GGT      50

             Met Val Glu Ala Asp Arg Pro Gly Lys Leu Phe Ile Gly
              1               5                  10

GGG CTT AAT ACG GAA ACA AAT GAG AAA GCT CTT GAA GCA GTA TTT GGC      98
Gly Leu Asn Thr Glu Thr Asn Glu Lys Ala Leu Glu Ala Val Phe Gly
    15                  20                  25

AAA TAT GGA CGA ATA GTG GAA GTA CTC TTG ATG AAA GAC CGT GAA ACC     146
Lys Tyr Gly Arg Ile Val Glu Val Leu Leu Met Lys Asp Arg Glu Thr
30                  35                  40                  45

AAC AAA TCA AGA GGA TTT GCT TTT GTC ACC TTT GAA AGC CCA GCA GAC     194
Asn Lys Ser Arg Gly Phe Ala Phe Val Thr Phe Glu Ser Pro Ala Asp
                50                  55                  60

GCT AAG GAT GCA GCC AGA GAC ATG AAT GGA AAG TCA TTA GAT GGA AAA     242
Ala Lys Asp Ala Ala Arg Asp Met Asn Gly Lys Ser Leu Asp Gly Lys
            85                  70  (A)             75

GCC ATC AAG GTG GAA CAA GCC ACC AAA CCA TCA TTT GAA AGT GGT AGA     290
Ala Ile Lys Val Glu Gln Ala Thr Lys Pro Ser Phe Glu Ser Gly Arg
        80                  85                  90

CGT GGA CCG CCT CCA CCT CCA AGA AGT AGA GGC CCT CCA AGA GGT CTT     338
Arg Gly Pro Pro Pro Pro Pro Arg Ser Arg Gly Pro Pro Arg Gly Leu
    95                  100                 105

AGA GGT GGA AGA GGA GGA AGT GGA GGA ACC AGG GGA CCT CCC TCA CGG     386
Arg Gly Gly Arg Gly Gly Ser Gly Gly Thr Arg Gly Pro Pro Ser Arg
110                 115                 120                 125

GGA GGA CAC ATG GAT GAC GGT GGA TAT TCC ATG AAT TTT AAC ATG AGT     434
Gly Gly His Met Asp Asp Gly Gly Tyr Ser Met Asn Phe Asn Met Ser
                130                 135                 140

TCT TCC AGG GGA CCA CTC CCA GTA AAA AGA GGA CCA CCA CCA AGA AGT     482
Ser Ser Arg Gly Pro Leu Pro Val Lys Arg Gly Pro Pro Pro Arg Ser
            145                 150                 155

GGG GGT CCT CCT CCT AAG AGA TCT GCA CCT TCA GGA CCA GTT CGC AGT     530
Gly Gly Pro Pro Pro Lys Arg Ser Ala Pro Ser Gly Pro Val Arg Ser
        160                 165                 170

AGC AGT GGA ATG GGA GGA AGA GCT CCT GTA TCA CGT GGA AGA GAT AGT     578
Ser Ser Gly Met Gly Gly Arg Ala Pro Val Ser Arg Gly Arg Asp Ser
    175                 180                 185

TAT GGA GGT CCA CCT CGA AGG GAA CCG CTG CCC TCT CGT AGA GAT GTT     626
Tyr Gly Gly Pro Pro Arg Arg Glu Pro Leu Pro Ser Arg Arg Asp Val
190                 195                 200                 205
```

Fig. 4

```
TAT TTG TCT CCA AGA GAT GAT GGG TAT TCT ACT AAA GAC AGC TAT TCA      674
Tyr Leu Ser Pro Arg Asp Asp Gly Tyr Ser Thr Lys Asp Ser Tyr Ser
                210                 215                 220

AGC AGA GAT TAC CCA AGT TCT CGT GAT ACT AGA GAT TAT GCA CCA CCA      722
Ser Arg Asp Tyr Pro Ser Ser Arg Asp Thr Arg Asp Tyr Ala Pro Pro
            225                 230                 235

CCA CGA GAT TAT ACT TAC CGT GAT TAT GGT CAT TCC AGT TCA CGT GAT      770
Pro Arg Asp Tyr Thr Tyr Arg Asp Tyr Gly His Ser Ser Ser Arg Asp
        240                 245                 250

GAC TAT CCA TCA AGA GAA TAT AGC GAT AGA GAT GGA TAT GGT CGT GAT      818
Asp Tyr Pro Ser Arg Glu Tyr Ser Asp Arg Asp Gly Tyr Gly Arg Asp
    225                 260                 265

CGT GAC TAT TCA GAT CAT CCA AGT GGA GGT TCC TAC AGA GAT TCA TAT      866
Arg Asp Tyr Ser Asp His Pro Ser Gly Gly Ser Tyr Arg Asp Ser Tyr
270                 275                 280                 285

GAG AGT TAT GGT AAC TCA CGT AGT GCT CCA CCT ACA CGA GGG CCC CCG      914
Glu Ser Tyr Gly Asn Ser Arg Ser Ala Pro Pro Thr Arg Gly Pro Pro
                290                 295                 300

CCA TCT TAT GGT GGA AGC AGT CGC TAT GAT GAT TAC AGC AGC TCA CGT      962
Pro Ser Tyr Gly Gly Ser Ser Arg Tyr Asp Asp Tyr Ser Ser Ser Arg
            305                 310                 315

GAC GGA TAT GGT GGA AGT CGA GAC AGT TAC TCA AGC AGC CGA AGT GAT     1010
Asp Gly Tyr Gly Gly Ser Arg Asp Ser Tyr Ser Ser Ser Arg Ser Asp
        320                 325                 330

CTC TAC TCA AGT GGT CGT GAT CGG GTT GGC AGA CAA GAA AGA GGG CTT     1058
Leu Tyr Ser Ser Gly Arg Asp Arg Val Gly Arg Gln Glu Arg Gly Leu
    335                 340                 345

CCC CCT TCT ATG GAA AGG GGG TAC CTC CTC CAC GTG ATT CCT ACA GCA     1106
Pro Pro Ser Met Glu Arg Gly Tyr Leu Leu His Val Ile Pro Thr Ala
350                 355                 360                 365

GTT CAA GCC GCG GAC GAC CAA GAG GTG GTG GCC GTG GAG GAA GCC GAT     1154
Val Gln Ala Ala Asp Asp Gln Glu Val Val Ala Val Glu Glu Ala Asp
                370                 375                 380

CTG ATA GAG GGG GAG GCA GAA GCA GAT ACT AGA AAC AAA CAA AAC TTT     1202
Leu Ile Glu Gly Glu Ala Glu Ala Asp Thr Arg Asn Lys Gln Asn Phe
            385                 390                 395

GGA CCA AAA TCC CAG TTC AAA GAA ACA AAA AGT GGA AAC TAT TCT ATC     1250
Gly Pro Lys Ser Gln Phe Lys Glu Thr Lys Ser Gly Asn Tyr Ser Ile
        400                 405                 410
```

Fig. 4 (cont.)

```
ATA ACT ACC CAA GGA CTA CTA AAA GGA AAA ATT GTG TTA CTT TTT TTA    1298
Ile Thr Thr Gln Gly Leu Leu Lys Gly Lys Ile Val Leu Leu Phe Leu
    415                 420                 425

AAT TCC CTG TTA AGT TCC CCT CCA TAATTTTTAT GTTCTTGTGA GGAAAAAAGT   1352
Asn Ser Leu Leu Ser Ser Pro Pro
430                 435

AAAACATGTT TAATTTTATT TGACTTCTGC ATTGCTTTTC AACAAGCAAA TGTTAAATGT   1412

GTTAAGACTT GTACTAGTGT TGTAACTTTC CAAGTAAAAG TATCCCCTAA AGGCCACTTC   1472

CTATCTGATT TTTCCCAGCA AATGAGGCAG GCAATTCTAG TCTTCCACAA AACATCTAGC   1532

CATCTAAAAT GGAGAGATGA ATCATTCTAC CTATACAAAC AAGCTAGCTA TTAGAGGGTG   1592

GTTGGGGTAT GCTACTCATA AGATTTCAGG GTGTCTTCCA ACTGAAATCT CAATGTTCTC   1652

AGTACGAAAA ACCTGAAATC ACATGCCTAT GTAAGGAAAG TGCTATTCAC CCAGTAAACC   1712

CAAAAAAGCA AATGGATAAT GCTGGCCATT TTGCCTTTCT GACATTTCCT TGGGAATCTG   1772

CAAGAACCTC CCCTTTCCCT TCCCCCAATA AGACCATTTA AGTGTGTGTT AAACAACTAC   1832

AGAATACTAA GTAAAAAGTT TGGCCAAAAC CAAAAAAAAA AAAAAAAAAA AAAAAAAAAA   1892

AA                                                                  1894
```

Fig. 4 (cont.)

Ⓐ

```
GCTATAGCAG AACCGCTGGG GTAACAACAA CCGGGATAAC AACAACTCCA ACAACAGAGG    60
CAGCTACAAC CGGGCTCCCC AGCAACAGCC GCCACCACAG CAGCCTCCGC CACCACAGCC   120
ACCACCCCAG CAGCCACCGC CACCACCCAG CTACAGCCCT GCTCGGAACC CCCCAGGGGC   180
CAGCACCTAC AATAAGAACA GCAACATCCC TGGCTCAAGC GCCAATACCA GCACCCCCAC   240
CGTCAGCAGC TACAGCCCTT CCACAGCCGA GTTACAGCCA GCCACCCTAC ANCCAGGGGA   300
GGTTACAGCC AGGGTTACAC AGG                                           323
```

Fig. 5

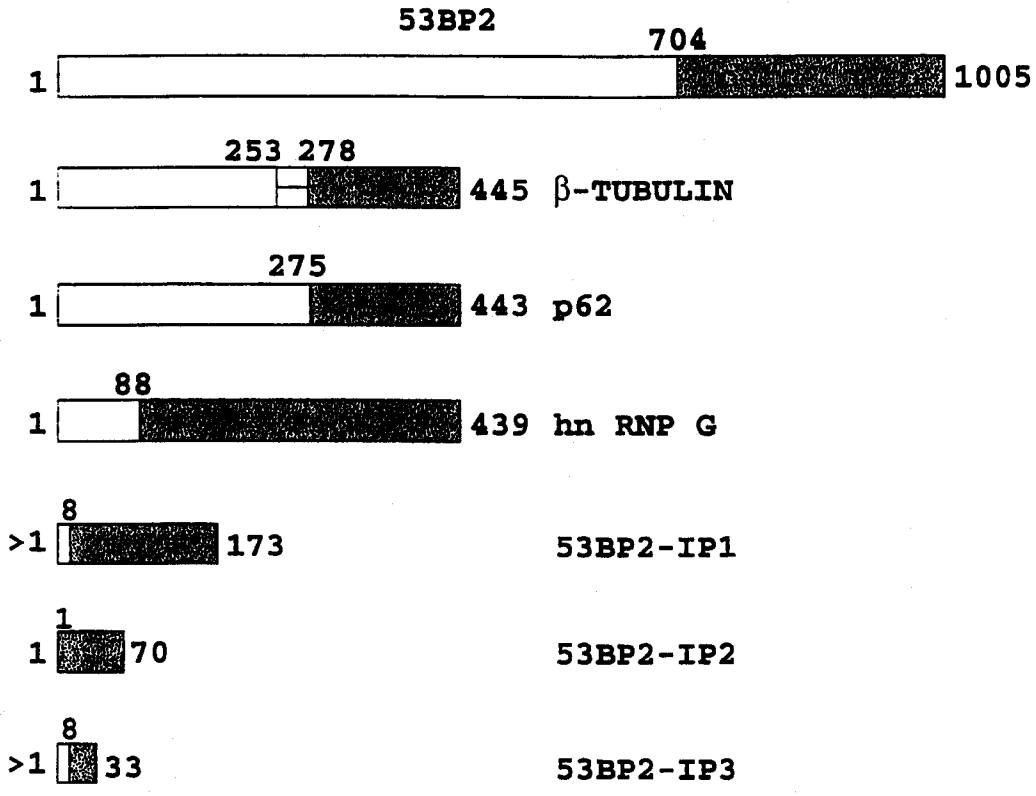

53BP2
704
1
1005
253 278
1
445 β-TUBULIN
275
1
443 p62
88
1
439 hn RNP G
8
>1
173
53BP2-IP1
1
1
70
53BP2-IP2
8
>1
33
53BP2-IP3

Fig. 6

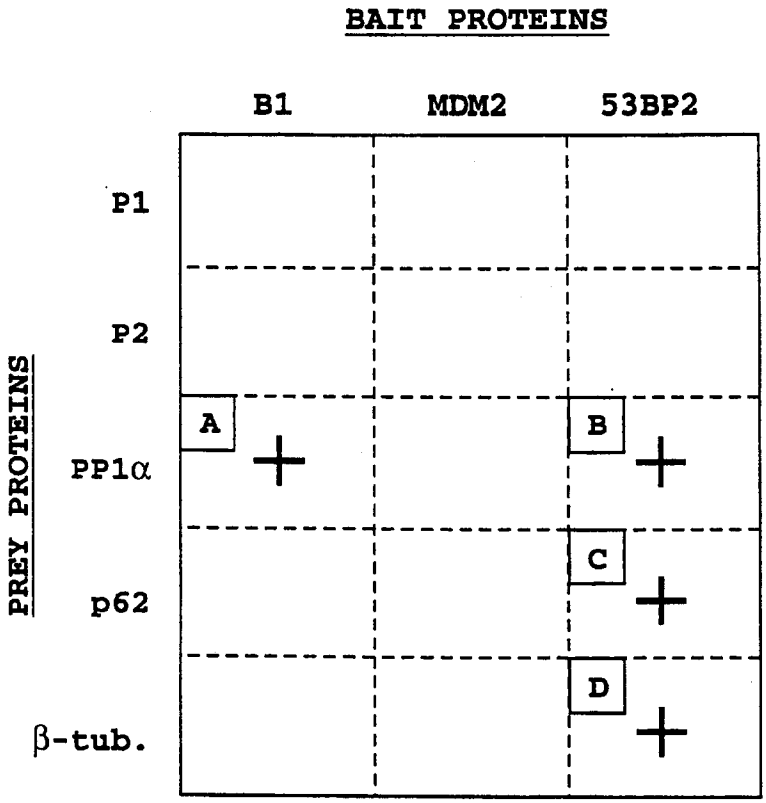

BAIT PROTEINS
B1
MDM2
53BP2
PREY PROTEINS
P1
P2
PP1α
p62
β-tub.
A
B
C
D

Fig. 7

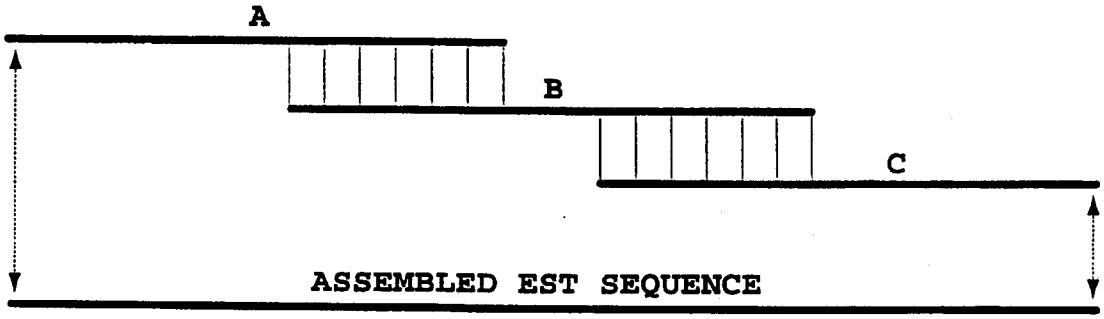
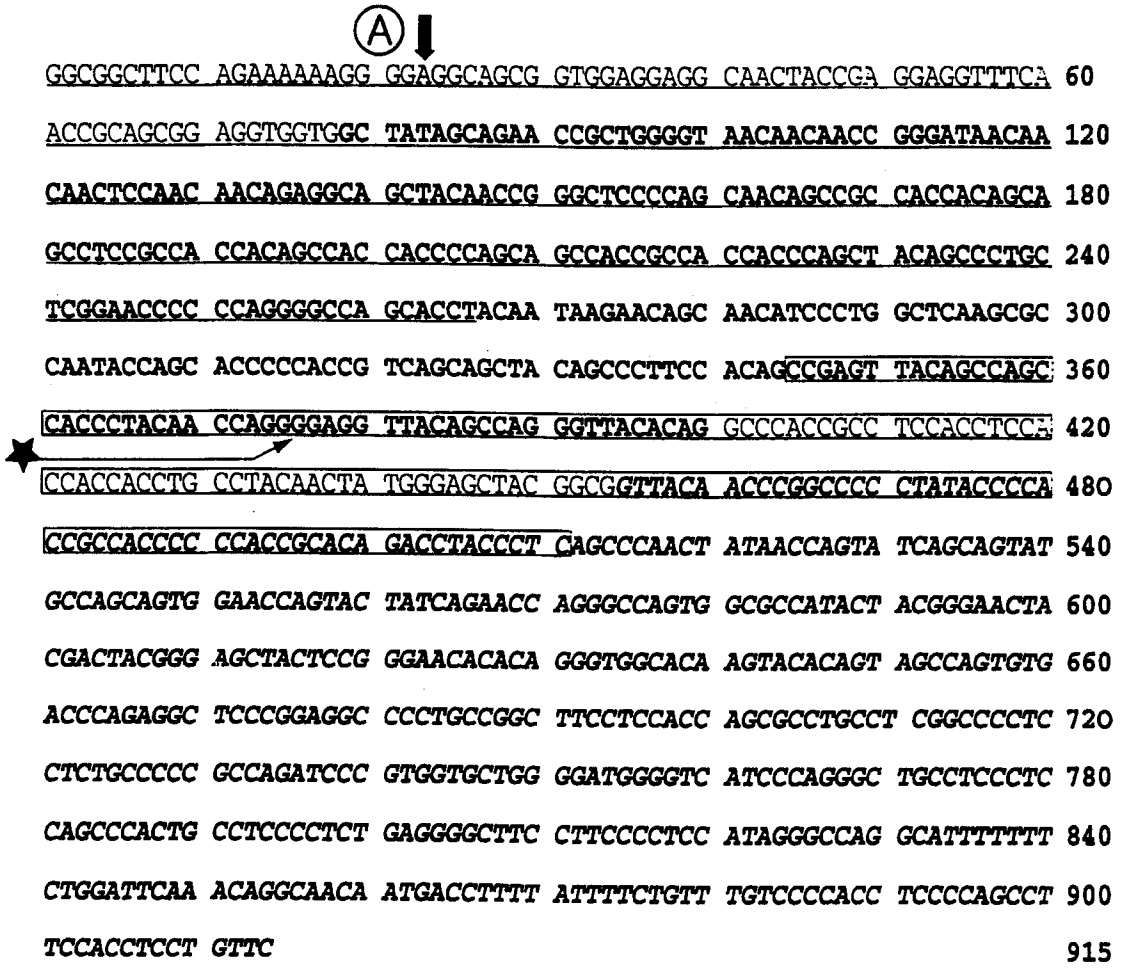

A
B
C
ASSEMBLED EST SEQUENCE

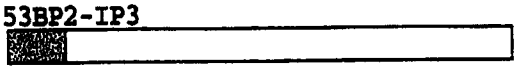
Fig. 8



EST C17385-DERIVED SEQUENCE	bold underline
EST R72810-DERIVED SEQUENCE	bold lettering
EST AA464793-DERIVED SEQUENCE	boxed lettering
EST AA479761-DERIVED SEQUENCE	bold italic lettering

Fig. 9

A. 53BP2-IP3



Translation Frame +1 1-102

1 GGC GGC TTC CAG AAA AAA GGG GAG GCA GCG GTG GAG GAG GCA ACT
Gly Gly Phe Gln Lys Lys Gly Glu Ala Ala Val Glu Glu Ala Thr

B. 46 ACC GAG GAG GTT TCA ACC GCA GCG GAG GTG GTG GCT ATA GCA GAA
Thr Glu Glu Val Ser Thr Ala Ala Glu Val Val Ala Ile Ala Glu

91 CCG CTG GGG TAA 102
Pro Leu Gly *

C. 53BP2-IP2

Translation Frame +2 440-652

440 ATG GGA GCT ACG GCG GTT ACA ACC CGG CCC CCT ATA CCC CAC CGC
Met Gly Ala Thr Ala Val Thr Thr Arg Pro Pro Ile Pro His Arg

485 CAC CCC CCA CCG CAC AGA CCT ACC CTC AGC CCA ACT ATA ACC AGT
His Pro Pro Pro His Arg Pro Thr Leu Ser Pro Thr Ile Thr Ser

D. 530 ATC AGC AGT ATG CCA GCA GTG GAA CCA GTA CTA TCA GAA CCA GGG
Ile Ser Ser Met Pro Ala Val Glu Pro Val Leu Ser Glu Pro Gly

575 CCA GTG GCG CCA TAC TAC GGG AAC TAC GAC TAC GGG AGC TAC TCC
Pro Val Ala Pro Tyr Tyr Gly Asn Tyr Asp Tyr Gly Ser Tyr Ser

620 GGG AAC ACA CAG GGT GGC ACA AGT ACA CAG TAG 652
Gly Asn Thr Gln Gly Gly Thr Ser Thr Gln *

E. 53BP2-IP1

Translation Frame +3 3-524

3 CGG CTT CCA GAA AAA AGG GGA GGC AGC GGT GGA GGA GGC AAC TAC
Arg Leu Pro Glu Lys Arg Gly Gly Ser Gly Gly Gly Gly Asn Tyr

F. 48 CGA GGA GGT TTC AAC CGC AGC GGA GGT GGT GGC TAT AGC AGA ACC
Arg Gly Gly Phe Asn Arg Ser Gly Gly Gly Gly Tyr Ser Arg Thr

93 GCT GGG GTA ACA ACA ACC GGG ATA ACA ACA ACT CCA ACA ACA GAG
Ala Gly Val Thr Thr Thr Gly Ile Thr Thr Thr Pro Thr Thr Glu

Fig. 10

```
138 GCA GCT ACA ACC GGG CTC CCC AGC AAC AGC CGC CAC CAC AGC AGC
    Ala Ala Thr Thr Gly Leu Pro Ser Asn Ser Arg His His Ser Ser

183 CTC CGC CAC CAC AGC CAC CAC CCC AGC AGC CAC CGC CAC CAC CCA
    Leu Arg His His Ser His His Pro Ser Ser His Arg His His Pro

228 GCT ACA GCC CTG CTC GGA ACC CCC CAG GGG CCA GCA CCT ACA ATA
    Ala Thr Ala Leu Leu Gly Thr Pro Gln Gly Pro Ala Pro Thr Ile

273 AGA ACA GCA ACA TCC CTG GCT CAA GCG CCA ATA CCA GCA CCC CCA
    Arg Thr Ala Thr Ser Leu Ala Gln Ala Pro Ile Pro Ala Pro Pro

318 CCG TCA GCA GCT ACA GCC CTT CCA CAG CCG AGT TAC AGC CAG CCA
    Pro Ser Ala Ala Ser Ala Leu Pro Gln Pro Ser Tyr Ser Gln Pro

363 CCC TAC AAC CAG GGG AGG TTA CAG CCA GGG TTA CAC AGG CCC ACC
    Pro Tyr Asn Gln Gly Arg Leu Gln Pro Gly Leu His Arg Pro Thr

408 GCC TCC ACC TCC ACC ACC ACC TGC CTA CAA CTA TGG GAG CTA CGG
    Ala Ser Thr Ser Thr Thr Thr Cys Leu Gln Leu Trp Glu Leu Arg

453 CGG TTA CAA CCC GGC CCC CTA TAC CCC ACC GCC ACC CCC CAC CGC
    Arg Leu Gln Pro Gly Pro Leu Tyr Pro Thr Ala Thr Pro His Arg

498 ACA GAC CTA CCC TCA GCC CAA CTA TAA 524
    Thr Asp Leu Pro Ser Ala Gln Leu *
```

Fig. 10 (cont.)